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THE JOURNAL OF PARASITOLOGY

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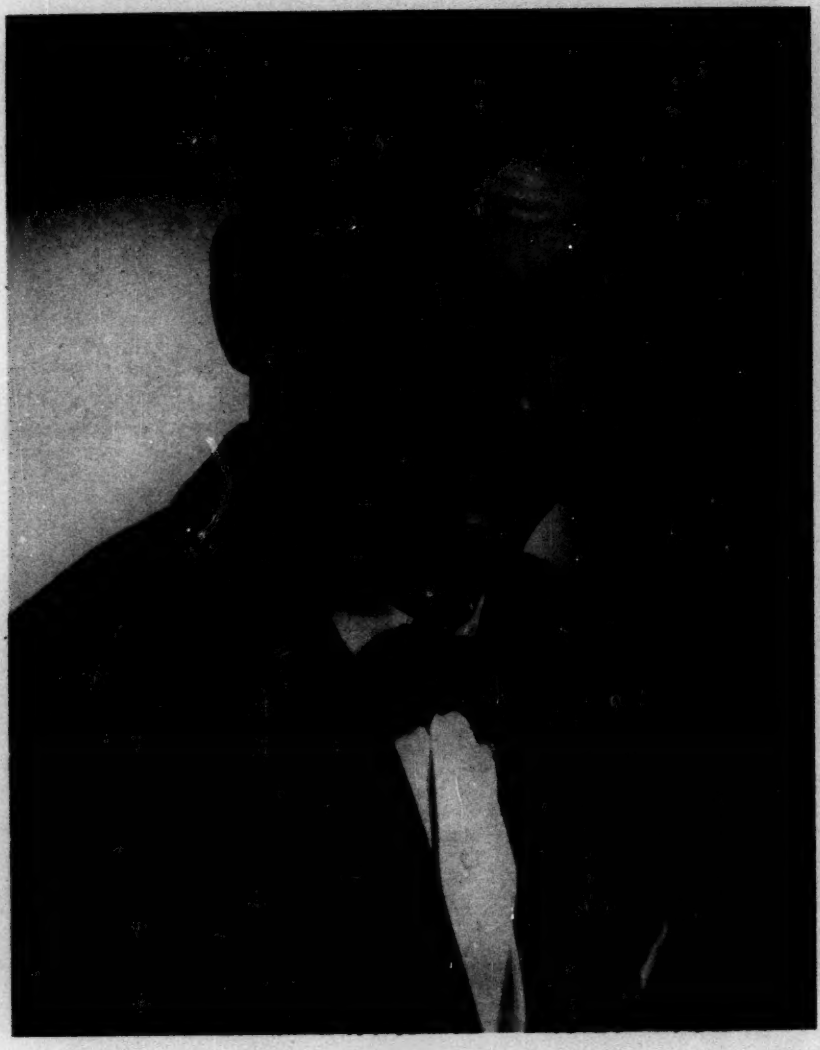

Gilbert F. Otto

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SOME REFLECTIONS ON THE ECOLOGY OF PARASITISM*

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Ecology may be conveniently defined as the science which deals with the relationship of an organism to and with its environment. My visualization of this concept is one of a dynamic inter-relation. Perhaps we could borrow a phrase from the physical chemist and refer to this as a dynamic fluctuating equilibrium. This would include the effects of the environment on the organism in all aspects of its life, on its metabolism, on its reproduction, its continuing evolution or survival as an individual, a clone, or a species. It would include the response of the organism, individually and as a group, to a changing environment and, equally true, the response of the environment to the changing conditions imposed by the organism. We are accustomed to apply the term ecology to the relation between free-living organisms and their environment. One speaks readily enough of the ecology of mosquitoes or of the ecology of the free-living hookworm larvae. Should we not in like manner consider the ecology of the malaria parasite in the mosquito or the ecological evolution of the hookworm as it moves from its free-living through its several environments in the mammalian host on its way to the small intestine?

Probably there would be rather general acceptance of a statement that the ecology of a parasite, particularly one which requires two or three different species of hosts in its ontogeny, is more complex than the ecology of a free-living organism. Yet I wonder if this is really true. Is the septic tank or the pond in which the ciliate lives any less complex than the alimentary canal in which the flagellate lives? Do the two hosts of the malaria parasite offer any wider variety of environmental factors than that which the salmon encounters in its migration from its many haunts in the ocean depths to its spawning beds above the rapids of the river? It is not my purpose to dwell on such comparisons or even attempt to answer the questions I pose. I raise these questions merely as the stepping stones for my more immediate question—do we adequately consider the parasite in its ecological relations? Have we adequately considered the ecological significance of the many diverse facets of information which have been provided by the comparative taxonomist, the ontogenist, the physiologist, the enzyme chemist, the immunologist, the immunochemist, the pathologist, the experimental nutritionist, the epidemiologist, and the clinician? When I see, in a modern day textbook, the epidemiology of hookworm disease confined to the simple statement that eggs are passed in the feces, development takes place in sandy soil, and infection is by way of skin penetration, I wonder. When I further note in contrast that paragraphs

* Address of the retiring President of the American Society of Parasitologists, 32nd Annual Meeting, Philadelphia, Pennsylvania, November 1, 1957.

are required to give the detailed measurements of the several stages, sharp time schedules for the several ecdyses, and endless detail on time spent in various parts of the body, my doubts increase.

Parasitology, as well as such associated sciences as clinical medicine, both human and veterinary medicine, has passed through the purely descriptive phase. We have been moving forward in the areas of experimental physiology, pathology, immunology, epidemiology, and chemotherapy along very broad fronts. And yet, we have not left the morphological phase behind us. Indeed the morphological and taxonomic requirements are becoming increasingly more rigorous, and the recognition of the organism is a necessary base line from which to interpret the experimental data. Taxonomy, on a morphological basis, is under continuing surveillance as a result of problems raised in epidemiology, clinical medicine, and experimental observation. A few examples will serve to illustrate the basic concepts I have in mind.

AMEBIASIS

The current confusion on amebiasis illustrates the need to fill in the gaps in our knowledge and the need for a more adequate ecological evaluation based on those data which are available. Two rhetorical questions will serve as a basis for consideration; these are (1) Can we recognize *Entamoeba histolytica* and do we know what constitutes this species? and (2) Is amebiasis ecologically a tropical and sub-tropical disease or is it better equipped for survival in the arctic and temperate zones? It is not my purpose here to answer questions, nor do I presume to answer these. On the contrary, I wish to emphasize the areas which appear to me to require further elucidation. Nevertheless, on the basis of the available experimental data and on the basis of my epidemiological observations, I have developed several working postulates. One of these is that we probably have not yet determined what constitutes the species known as *Entamoeba histolytica*, the causative agent of amebiasis; that we do not understand its ecological relations within and with its human host. Another working postulate is that the organism *per se* is better fitted for survival and transport in the external environment in the temperate zones, perhaps even the arctic, than in the tropics and that water is probably not a particularly effective vehicle for transportation of the cysts to the next victim. These latter postulates have developed in the face of my recognition that clinical amebiasis is far more common in the tropics than in any other area of the world and that there have been several epidemics of amebiasis which quite evidently were water-borne. But let us first go back to the question of the species involved and the ecological relations within the hosts.

It is probable that the pathogenic ameba of man was first seen in the stools of dysenteric patients in India, but the organism we now know as *Entamoeba histolytica* Schaudinn, 1903, was first recognized and described in Russia by Lösch in 1875 as *Amoeba coli*. Incidently, it was Lösch who first experimentally infected dogs and produced the disease in one of four animals. Perhaps because typical disease developed in only one of the animals, and anticipating some of the current evidence, he concluded that the parasites alone did not produce the disease. In fact, he went to the extent of suggesting that the amebae merely prevented the healing of the ulcerations from some other primary etiology. During the next forty years many workers have contributed to the description and the differentia-

tion of this organism from harmless commensals, including Craig's experimental infection of kittens in 1905 to differentiate *E. histolytica* from *E. coli.* Any doubt which might be harbored on the validity of this distinction was eliminated by Walker and Sellards' (1913) report of the results of their experimental infection of human subjects. It is, however, germane to our discussion today to recall that these workers actually produced infection in only 18 of 20 patients and that only four developed recognized disease manifestations, a point which will be considered later. Over the years certain apparently well-defined species have been distinguished from *E. histolytica,* such as *Entamoeba coli* (Grassi, 1879) Casagrandi and Barbagello, 1895; *Iodomoeba bütschlii* (v. Prowazek, 1912) Dobell, 1919; *Endolimax nana* (Wenyon and O'Connor, 1917) Brug, 1918; and *Dientamoeba fragilis* Jepps and Dobell, 1918. A troublesome ecological and taxonomic problem involving the *E. histolytica* complex with its four-nucleate cysts still remains. The troublesome question of the small race, races, or species is yet to be wholly resolved. Even before Walker and Sellards had experimentally distinguished the non-pathogenic *E. coli,* the spector of a small race of *E. histolytica* had appeared (*Entamoeba minuta* Elmassian, 1909), and *Entamoeba hartmanni* Prowazek, 1912, was reported shortly thereafter. These and the subsequently described small species and other variants from the pathogenic dysentry ameba have been synonymized by Brumpt, in his Précis de Parasitologie, under the species names *dispar* and *minuta.* Many workers have contributed to our knowledge of the morphology of these forms. These contributions have been reviewed recently by Hoare (1952, 1957) and Burrows (1957) who present a strong case for *E. hartmanni* Prowazek, 1912, which antidates *E. dispar* Brumpt, 1925, as a non-pathogenic species morphologically distinct from *E. histolytica* and with a smaller four-nucleate cyst than the latter. It implies no criticism of these studies, or the conclusions derived from them, to point out that the average clinical laboratory will encounter difficulty in utilizing these morphological criteria in the practical separation of *E. hartmanni* from *E. histolytica.* The difficulties encountered in attempting to develop unequivocal characters for the practical recognition of the complex, which we have known as *E. histolytica,* have been set forth recently (Brooke *et al,* 1953). One can only speculate on the additional complications involved in the attempts to distinguish *E. hartmanni* and *E. histolytica* in the clinical laboratory.

The problem is further complicated from the standpoint of the clinical laboratory by the suggestion of a small, *"minuta"*, trophozoite of *E. histolytica, stricto sensu,* which lives as a commensal. This focuses directly on the ecology of the dysentery ameba in its human host. You will recall that Dobell was one of the early exponents of the postulate that *E. histolytica* was an obligate tissue parasite in its trophozoite stage and that Craig has urged in his many contributions that there is tissue penetration in every infected individual; in short, that there is biologically no such thing as a true "carrier" infection with *E. histolytica.* The concept of a commensal phase in the life cycle is in direct conflict with the concept of an obligatory parasite. As early as 1913, Kuenen and Swellengrebel postulated a commensal phase as an integral and essential part of the life cycle of this organism. Hoare (1952, p. 424) concludes that only the commensal, or *"minuta"* trophozoites are capable of producing cysts. Thus the completion of the life cycle would not require or even include the tissue-invading trophozoites. Tissue invasion is pic-

tured as occurring only under exceptional circumstances when the ecological conditions in the colon either render the trophozoites more virulent or reduce the normal resistance of the tissues to invasion. Although Hoare in 1952 concluded that only the commensal, or *"minuta"* trophozoites can produce cysts, the discussion preceding the conclusion is less sharply focused, and in 1957 his diagram of the life cycle of *E. histolytica* includes the formation of cysts not only by the commensal trophozoites, or *"minuta"* forms but by the larger tissue-invading trophozoites as well. It is an interesting area for speculation and offers a fruitful opportunity for experimental exploration.

It would be presumptious of me to attempt to arbitrate these several conflicting theories, nor will I develop any series of arguments in favor of any one aspect. Nevertheless, I confess a fascination for Sir Cecil Hoare's main premise. Ecologically, it offers explanations for much that is unexplained by the "obligatory parasite" concept. Yet, I can accept them as no more than well developed theories or working postulates. An Euclidian theorem is amenable to mathematical proof; a biological theory, however well developed, remains to be substantiated by additional observations and experimentation. In the meantime, the task before the clinical laboratory is exacting but confusing, i.e., does the patient harbor a pathogen, a potential pathogen, or a harmless commensal, and if so, how does one know?

Before leaving the question of the species involved in the production of amebic dysentery, attention may be directed to a few other observations. Regardless of whether different species, physiological varieties, or merely phases in the life cycle are involved, the evidence urges that amebic colitis is associated with a large tissue-invading trophozoite and a fairly large (±12 microns) four-nucleate cyst. Presumably, it is also this large trophozoite that is associated with amebic hepatitis. Conversely, a smaller trophozoite and a smaller four-nucleate cyst (±8 microns) is associated with asymptomatic infections (Spector, 1936; Sapero, Hakansson, and Louttit, 1942). Furthermore, Sapero and his co-workers report that the size of the cyst remained the same throughout many months of observation, during clinical periods of remission and relapse. In general, the smaller race has proved more difficult to culture and upon inoculation into kittens, weanling rats, or rabbits has produced less pathology than the large race. Hunninen and Boone (1957) found that small strains and some of the large strains in their series colonized in the lumen of rabbits without any evidence of tissue invasion. Only five of their two dozen or more large strains regularly produced severe pathology in the rabbits; four were from clinical cases of amebiasis, and one was from an asymptomatic carrier. Some tissue invasion and pathology has occurred with small races in kittens (Frye and Meleney, 1938) and in rats (Neal, 1957). However, kittens, weanling rats, or young rabbits hardly provide the same ecological environment as an adult human so that we cannot consider this as unequivocal demonstration that these small strains would have the same capacity to invade the tissues of the human colon. Recently, Beaver *et al* (1956) reported the infection of 42 human volunteers with a strain from an asymptomatic carrier, "morphologically typical of large race *E. histolytica* (which) established vigorous colonies in standard culture media". Although the infection became established in 100% of the volunteers, none of them developed any clinical evidence of amebiasis. Nevertheless, dysentery supported by post-mortem evidence of tissue invasion occurred in 2 dogs

which became infected out of 10 exposed. Likewise, five of 18 guinea pigs became infected with mild to severe lesions demonstrated on post-mortem examination. Mild lesions were demonstrated in only four of the ten rats which were exposed and became infected.

On the other hand, more severe lesions were demonstrated in both rats and guinea pigs infected with a strain isolated from a clinical case of amebiasis.

Even more recently, Shaffer, Connor, and Rosenthal (1957) have found evidence that the metabolism of two small races was quite different from that of two large races. The large races grew readily on egg slants with an overlay of Locke's solution without penicillin or with penicillin G (maximum used, 1316 units/ml). Whereas, the two small races were both inhibited by penicillin G and many degenerating forms had the superficial appearance of *Blastocystis hominis*. Furthermore, the cultural and nutritional requirements of the two small races appeared to differ one from the other. One strain (Griffith) grew readily on the above media but was inhibited by as little as 164 units/ml of penicillin G. It did not grow well on a liver slant medium. The second strain (Conrad) would not grow on the egg slants at all, even in the absence of penicillin. It grew well on a liver slant overlaid with human serum-Locke's solution but in this medium was inhibited by as little as 82 units/ml of penicillin G and at 132 units the degenerating *Blastocystis*-like structures appeared. In addition to the obvious indication of metabolic and ecological differences between the large and small races and probable differences in response to therapeutic agents, a number of other questions are raised. For instance, what relation is there between these *Blastocystis*-like degenerating ameba and the *Blastocystis hominis* so commonly found in human feces? If there are two biologically distinct trophozoites in the *Entamoeba histolytica* cycle, a commensal trophozoite and a tissue-invading trophozoite, are the metabolic pathways of the two so different that they will have fundamentally different responses to therapeutic agents?

If, at this point, you know precisely what constitutes the species *Entamoeba histolytica* and can infallibly recognize the dysentery-producing ameba, your knowledge preceded this discussion. I am not so fortunate, or perhaps more accurately, not so unfortunate. I have only some working postulates to guide me. I cannot escape the premise that what is usually called *E. histolytica* is a complex of at least strains and more probably species. I find the reported species, *Entamoeba hartmanni* intriguing. The concept that *E. histolytica, stricto sensu,* has a basic commensal cycle, with intermittent pathogenetic trophozites developing therefrom, is even more stimulating.

Involved in this latter picture, however, is a biological and ecological aspect that is not usually discussed. The biological analogies make it difficult for me to believe that the cyst serves solely as resistant stage, i.e., resistant to the external environment in its passage from one host to another. It has appeared to me probable that the cyst stage serves also as the locus of a biological rejuvenation which would most likely include nuclear and chromosome reorganization—i.e., at least the biological precursor of a sexual process. Accordingly, it would appear likely that cyst-formation might revitalize a strain. Support for this concept appeared to be provided by Chang (1945). Chang found that two strains, which, as a result of many asexual divisions in culture, had lost their capacity to infect kittens,

regained their tissue invasiveness after encystment in culture. Unfortunately for the premise, this work has not been confirmed. On the contrary, Neal (1951, 1954, 1957) found that encystment in culture neither increased the invasiveness of non-pathogenic strains nor revitalized strains which had lost their invasiveness in culture. Furthermore, Neal found that recurrent encystment did not prevent the gradual loss of invasiveness. The full biological significance of the cyst remains to be clarified.

The only successful attempts to revitalize strains, the invasiveness of which had been lost or reduced in culture, has been by selective passage through susceptible animals. Repeated passage through the intestine of cats (Cleveland and Sanders, 1930) or dogs (Faust and Swartzwelder, 1935; Meleney and Frye, 1937; Chang, 1945; Thompson, McCarthy, and Reinertson, 1954) has consistently increased the invasiveness of devitalized cultures. Neal (1956a) found repeated passages of devitalized strains through the liver of hamsters would restore their capacity to invade and produce lesions in the caecum of rats. However, strains which on original isolation had no capacity for tissue invasion in rats did not acquire such capacity even after 10 passages through the hamster's liver. It should be borne in mind that only asexual multiplication in the trophozoite stage has been observed in such animal passages. It is difficult to visualize mutation or any genetic selection or reconstitution under these circumstances. Any selection involved would appear to be only an overgrowth of the non-invasive lumen dwellers in culture followed by the reverse in animal passage. Whether such selection occurs or whether capacity for invasion is altered in the organisms *per se* by nutritional changes is not clear.

It is pardonable, I hope, at this point to digress further from the question of the species involved, to consider a few additional points on the pathogenicity of the dysentery-producting ameba. Apart from the evidence, both in man and experimental animals, that pathogenic bacteria may contribute to the ultimate course of the disease, the role of the accompanying bacteria is yet to be clearly defined. It is generally accepted on the basis of the available evidence that the primary lesion in amebiasis is due to the invasiveness of ameba *per se*. Certainly the attempts to increase the tissue invasion by inoculating bacteria from invasive strains with either non-invasive strains or those which have lost their invasiveness in culture has not given unequivocal evidence that the bacteria increased the invasiveness (Stewart and Jones, 1948; Stewart, 1948; Neal, 1956; Neal and Vincent, 1956). Likewise, the inoculation of bacteria from non-evasive strains has not reduced the invasiveness of the pathogenic strains. That the bacteria play some role is clear, however, from the findings of Phillips, *et al*, (1955) that the amebae from ameba-trypanosome cultures could not even establish in germ-free guinea pigs without bacteria. However, the addition of either of two bacteria (*Aerobacter aerogenes* or *Escherichia coli*) not only permitted the amebae to become established in the otherwise sterile guinea pig, but the amebae invaded the tissues. Earlier studies by Luttermoser and Phillips (1952) had demonstrated reduced capacity of amebae from ameba-trypanosome cultures to establish in regular laboratory-raised guinea pigs; only one of 24 became infected. Amebae cultured with a single species of bacteria (organism *t*) produced only about 50% infection in laboratory-raised guinea pigs, whereas those cultivated with either of two mixed flora produced

about 90% infection. The extent of the pathology varied but not to the same degree as the infection rate. The attempt by Frye and Meleney (1933) to alter the pathogenicity of amebae by changes in associated bacteria suggested that the elimination of the bacteria by washing reduced infectivity for kittens but shifting from one mixed flora to another had no such effect.

Certainly bacteria play some, as yet undefined, role in the growth of *E. histolytica* in culture and probably in the colonization in the host. But whether bacteria play a direct role in tissue invasion in man or merely permit the amebae to colonize in the lumen, as in the culture, and thus afford them the opportunity for subsequent tissue invasion is yet to be resolved.

Thus it would appear that there are significant gaps in our knowledge of the taxonomy and biology of the dysentery-causing ameba. The comparative ecological relations, the similarities and differences, in the human host, in experimental animals, and in culture are not sharply delineated. Resolution of these questions is of fundamental biological interest. Furthermore, the questions are of practical significance. Specifically, such information would be of immediate value in the area of chemotherapy, both in the experimental evaluation of old and new therapeutic agents and in the utilization of such agents in clinical medicine.

The question of whether amebiasis is better provided for survival and transmission in the tropics or in cooler climates involves the consideration of several different ecological factors. It seems to me that there has been a too easy assumption that the organism is *per se* adapted for the tropics and again that it is commonly transmitted in the water supply. In general, both the infection rate and the prevalence of frank disease is highest in the tropics. But what does this signify with reference to the ecology of the parasite and the epidemiology of the disease?

On several occasions (Otto, 1951, 1955), I have called attention to the overwhelming evidence that the cysts of *E. histolytica* survive infinitely longer at freezing temperatures than at tropical temperatures. Filipchenko (1935) using three strains, including a strain from the tropics, reported that survival times were three months at –20°C (–4°F), one month at 0°C, 14–17 days at 13–17°C, and 24–48 hours at 34–37°C. The reported survival time at any given temperature has varied under different conditions and with different indices of survival. Boeck (1921) using the eosin-staining index of viability reported that cysts washed from feces and then placed in distilled water from 12–22°C, survived for five months, but if the preparation was sealed with vasoline, they survived seven months. Kuenen and Swellengrebel (1913) using the same index of survival reported survival in a feces-water medium with a heavy bacterial growth was only 9 days at 27°–30°C and 3 days at 37°C, whereas they were still alive after 29 days at 21°C with very little bacterial growth. Yorke and Adams (1926) using the culture test reported that 50°C was immediately lethal, 45° was lethal in 30 minutes but that 0°–5° was not injurious in 48 hours. Swartzwelder (1939) reported that cysts stored at 3°–6° for 43 days, followed by 15 hours at room temperatures, appeared granular and degenerate but produced infection when fed to a dog. Craig (1944) cites a report by Stone that cysts from culture were still viable after 14 months at 0°C. In spite of the variation shown in these and other reports, there does not appear to be any contradiction to the basic evidence that the cysts of

E. histolytica under any given set of conditions survive longest at the lower temperatures at least down to 0°C and probably somewhat lower. How do we reconcile the tropical distribution of this infection with these indications that the transfer stage, the cyst, is best equipped for survival and distribution in the colder climates? It seems to me that some indication is found in a comparison of prevalence rates in certain population units.

For this purpose, I have brought together in table I and figure 1 the prevalence rates reported in 5 different population groups in the United States and plotted them against the incidence of infection reported in the five water-borne epidemics

TABLE I. *Comparison of the incidence of* Entamoeba histolytica *infection in water-borne epidemics with the prevalence rates in various population groups in United States.*

Location	*Authority*	*% Infected*
A. Water-Borne Epidemics		
Mantetsu Apartments, Tokyo (American)	Ritchie and Davis (1948)	63
Chicago Firemen	Hardy and Spector (1935)	52
South Bend Factory	Brooke, et. al. (1955)	52
Chicago Hotel Employees	Bundesen, et. al. (1936)	(21–68) 40*
British Air Force Barracks	Morton, Stamm, & Seidelin (1952)	(2–11) 4*
B. Reservation Groups (American Indians)		
Wyoming (children)	Owen, Honess, & Simon (1934)	27
New Mexico	Spector and Hardy (1939)	26
C. Mental Hospitals		
Georgia	Reardon (1941)	40
Maryland (school for feeble-minded)	Armaghan and Mediary (1938)	14
New Jersey	Faust (1942)	14
South Carolina	Burrows (1943)	8
" "	Young and Ham (1941)	4
Southern Indiana	Hopp (1940)	3
Northern Indiana	Kmecza (1939)	1
D. Rural Populations		
Tennessee	Milam and Meleney (1931)	36
"	Meleney (1930)	17
"	Eyles and Jones (1954)	11
Georgia	Seckinger (1936)	21
Virginia	Faust (1930)	20
Mississippi	Eyles and Jones (1954)	11
Oklahoma	McMullen and Gray (1941)	10
Indiana	Headlee and Hopp (1940)	4
E. Orphanages		
Louisiana	Faust (1931)	13
Maryland	Boeck (1921)	11
Oregon	Owen (1932)	5
F. College Students		
New Orleans, Louisiana	Faust (1942a)	8
" " "	Swartzwelder (1938)	3
Athens, Georgia	Byrd (1937)	5
Berea, Kentucky	Headlee and Cable (1942)	5
Philadelphia Pennsylvania	Wenrich, Stabler, and Arnett (1935)	4
St. Louis, Missouri	Tsychiya and Jean (1940)	2
Dartmouth, New Hampshire	Connell and French (1939)	1

* The rates vary according to the degree of exposure.

recorded in the literature. The 5 population groups may be classified as residents in the environment under study. I have not knowingly excluded any survey within such groups, but I do not insist that I have not missed any. I have excluded selected populations, such as those in out-patient dispensaries, or doctor's offices or clinics, or any surveys among hospital transients, or military groups. The only selected populations included are the college students, the last group included. Three facts seem to stand out sharply: (1) The recognized and recorded water-borne epidemics have all occurred in northern communities, (2) The infection rate in 4 of these 5 water-borne epidemics is materially greater than that recorded in any of the resident population groups in United States, and (3) Most of the surveys on resident population groups have been in the South.

Water supplies have long been suspected of being the vehicle for the spread of amebiasis as with other enteric infections. None of these suspicions had been verified until the epidemic which originated in two Chicago hotels in 1933. This epidemic, together with subsequent water-borne epidemics, has been interpreted in many quarters as substantiating earlier suspicions. But, is this a justifiable conclusion or is it even a reasonable conclusion? I strongly suspect that this is merely an easy explanation for a series of complex situations for which finite data are lacking. Let us look at the situation in these water-borne epidemics. The first four involved direct contamination of the water supply in the immediate location

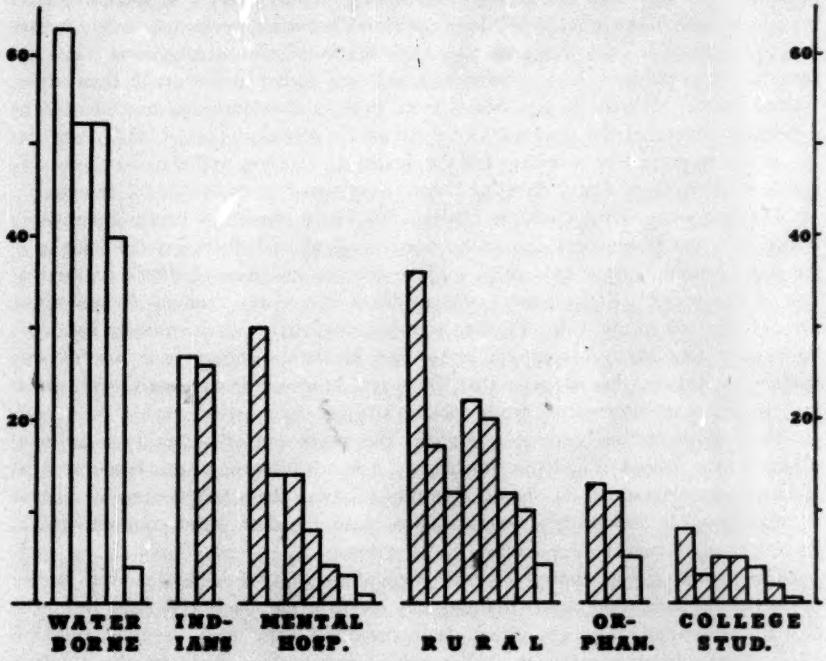

FIGURE 1. Comparison of the *Entamoeba histolytica* infection rates (percentages) in water-borne epidemics with those in various population groups in United States. The data are in the same order shown in table I. Open bars are northern communities; shaded bars are southern communities.

of use. The contamination was with fresh sewage, with the water in recurrent movement to keep the cysts and other particulate matter in suspension. In the Mantetsu apartments the drinking water had a leaky connection at a point where (1) it was submerged in sewage from the same apartments and (2) where it was under negative pressure in sucking the water (and sewage mixture) up into the storage tank on the roof. In the South Bend factory the situation was essentially the same—the water pipe submerged in sewage from the plant was under negative pressure of the pump sucking the water from the well and forcing it directly into the drinking fountains and other points of use. In the 2 Chicago hotels the contamination was guaranteed by multiple sources of fecal supply—i.e, dripping

of sewage into the ice water cooler, back siphonage from the commodes, and direct cross-connections between the water lines and the sewage lines. The Chicago firemen referred to here had recurrently quenched their thirst with the mixture of water and sewage used to fight the stocklard fire for 3 days. In these 4 epidemics there was continuous or intermittent *direct* contamination of moving water close to the point of use. Yet even under these circumstances, there is considerable evidence that infection of disease-producing levels was not easily acquired. The data available permit definite interpretation only in the case of the Chicago hotels with their multiple sources of water contamination. Even under these circumstances, the attack rate with clinical amebiasis overnight guests was only 3 in 1000, in guests for 7 to 14 days in the hotel 11 to 13 per thousand, but among semi-permanent guests (30 to 90 days) 111 per thousand, i.e., 11% developed clinical amebiasis. We are accustomed to think of the amebiasis rate as being higher in the orient than in the United States. It may be significant, then, that, in the Mantetsu apartments, the Americans living in the apartments developed an infection rate of 63%, whereas the Japanese domestics, working for the American families and thus in the apartments scarcely more than a third of a day, were found to be only 22% infected.

The fifth water-borne epidemic (British Air Force Barracks) was comparatively minor in terms of numbers and proportions involved and differs epidemiologically from the preceding four epidemics. There was no evidence of direct contamination of the water. Rather, there was evidence of seepage from a broken sewer through the soil to the well. Repairs were effected early. It is unlikely that contamination was at time comparable to that in the preceding four water-borne epidemics. Again, this suggests that the severe epidemic is associated with direct and continued or intermittent fresh contamination.

These data neither deny nor confirm the premise that water is a common vehicle in the spread of endemic amebiasis. They do suggest to me, however, that in highly endemic areas we should critically examine the water source in relation to other possible sources of infection before assuming that water contaminated at its source is the means of spreading the infection.

The fourth group shown (Rural Populations) gives evidence of a higher prevalence rate in the South than in the North. Although the data from the North are limited, they are directly supported by data from hospitals, medical clinics, and military personnel. All available information supports the evidence that amebiasis is more common in the southern United States than in the northern part of the country. Within the South there is, in general, a higher prevalence rate in the mountain and foothill areas than in the adjacent agriculturally more productive low lands. Even within the mountain and foothill areas there is evidence of further localization in certain families (Meleney, 1930; Eyles, Jones, and Smith, 1953; Mackie *et al*, 1955). Within this area there is a similar familial distribution of *Ascaris, Trichuris,* and hookworm (Otto, Cort, and Keller 1931). There is no evidence that flies, food-handlers, feces-fertilized vegetables, or water supplies play any part in the dissemination of these worm infections in these areas. On the contrary, the evidence is pretty clear that they cannot account for the basic distribution. Human feces is not used for fertilizer, in fact garden vegetables are unusual in much of the area. In the absence of modern toilet facilities and the indifferent use of such structures as may be available, there is a continuing supply

of fresh fecal material at the sites of immediate convenience close to or even within the house. Careful epidemiological reconnaissance disclosed that the water supply, spring or well, was usually removed some distance from the house and never, in my experience, has there been opportunity for gross fecal contamination of the water comparable to the concentrated contamination immediately around the house and only rarely was there possibility of an direct fecal contamination of the water supply.

The second group (Indian Reservations), one in the far North and the other in the arid Southwest, with the very high infection rates again are associated with uninhabited fecal contamination immediately around the premises. These communities represent a step forward in favor of the dissemination of the parasites. Fecal contamination is concentrated among a group of houses in an area of population concentration in contrast to such contamination around the individual rural home in the preceding group.

The level of sanitation and extent of the fecal contamination in the institutional group is a function of the type of inmates and the extent of facilities and the supervision rather than a function of the geographical location. With some mental patients the living quarters and exercise areas are recurrently contaminated with fecal material with little or no regard for the sanitary provisions or regulations. Burrows (1943) presents evidence that even in South Carolina the patients on admission have a low rate of infection (less than 2%) and that the infection rate builds up over the years to the 8% or more shown in the table. Orphanages, in general, are much better, with breaches in the sanitary code less obnoxious. In either type of institution the opportunity for dissemination of such intestinal parasites as *E. histolytica* from any breach in sanitary principle is increased by virtue of increased opportunity for human contact with the infective material over that which occurs in the dooryard of of single family. In any such institution any breach in the sanitary code offers opportunity for disseminating the infection without use of the drinking water system and, in most installations, it is impossible to visualize any contamination of water supply.

Several years ago, through the courtesy of Dr. H. W. Brown, I had the opportunity to see in a northern State a situation in an institution for mentally defective children which sharply illustrates the role of a mass fecal contamination in the spread of amebiasis. Among a group of approximately 100 toddlers, few escaped infection and as high as 35% were acutely ill with amebiasis at one time. These children were physically active and played together in one large room, except when prevented by acute illness. Due to their age and mental deficiencies, the children were very careless in their personal habits and, in spite of the efforts of attendants, the play room floor and the children themselves were continuously being contaminated with feces. In contrast, amebiasis was uncommon among the 100 children who were permanently bed-fast in an adjacent room in the same building but who had no direct contact with the toddler group. Food, water, and food-handlers are ruled out as the primary sources of infection since both groups were fed from a common kitchen, and attendants common to both groups were known to be infected.

Finally, the groups of college students (F.) illustrate that in the economic, social, and age groups from which college students are drawn, there is com-

paratively little infection. Perhaps it is a bit higher among those in southern schools than in northern schools but certainly the difference is not great.

The human species is not instinctively sanitary. Any parents among you can testify that it is necessary to "house-break" a child just as it is necessary to house-break a puppy. The late Charles Wardell Stiles, in his picturesque language, pointed out that the human race had evolved basically only to the dog stage of sanitation in contrast to more advanced sanitary evolution of the cat which instinctively buries its feces. The modern efforts to improve urban and rural sanitation through legislation had their earliest counter-part in the Mosaic law. We might even have considered as our text today, Deuteronomy 23:12 and 13:

> "Thou shalt have a place also without the camp, whither thou shall go forth abroad:
> "And thou shalt have a paddle upon thy weapon; and it shall be, when thou wilt ease thyself abroad, thou shalt dig therewith, and shalt turn back and cover that which cometh from thee."

This serves as an excellent focal point in considering the ecology of any feces-borne infection. Aesthetic considerations, even more than specific attempts at disease control, have led individuals and communities to make provisions for fecal disposal from ancient times to the present day. Superimposed on this cultural development has been added many technical features specifically designed to reduce or eliminate the spread of infectious material in feces. In many areas, notably in the orient, the economic value of "nightsoil" has added a complication both from the aesthetic and disease control standpoints. These are completely artificial procedures, rather than natural attributes of man. Each successive generation must be taught to develop, maintain, and use the facilities. Such teaching continues both on a family or personal level and on a community level. In northern communities, with inclement weather during much of the year, the aspect of personal comfort provides additional inducement in the learning process. Apparently, in the economically less fortunate groups, less effort is expended in "housebreaking" the unsanitary human child than most of us in this room expend. Thus, without the pressure of inclement weather, particularly among population units with little or no contact with modern methods of fecal disposal, in warmer climates many such families have never developed artificial provisions for fecal disposal. Such families will, at most, make indifferent use of facilities provided for them by public agencies or by industrial housing developments. Among the economically less fortunate groups, it is very difficult to induce the individual to expend funds or energy for the construction of sanitary facilities rather than for other comforts or necessities. Sociologists and economists have noted that, in general, there is a denser population of marginal lands in warmer climates than in colder climates. Thus, the ecology of man has helped develop more favorable opportunities for the dissemination of such enteric infections as amebiasis in the tropics and subtropics. In such areas, including southern United States, there are large population units, mostly rural or semi-rural, in which the accepted pattern of fecal disposal is at the site of immediate convenience. This provides a continuing supply of fresh feces close to the house.

Nothing as subtle as the infected food handler is required to explain the familial distribution of amebiasis under such circumstances. Under these circumstances, contamination of water, particularly in wells, is not only possible but probable.

However, the contamination of wells, cisterns, or springs would be largely by seepage and drainage and often these water sources are some distance from the house. Unsatisfactory as they may be from a good sanitary standpoint, they are less exposed to a continuing supply of fresh fecal material than is the individual himself, working, playing, or otherwise relaxing in or around the house. When amebiasis is endemic under such conditions, I strongly suspect that the effective treatment of food-handlers, destruction of flies, and installation of a contamination-free source of drinking water would not have any discernible effect upon the endemicity of amebiasis, unless it directly or indirectly improved the matter of fecal disposal. The difficulty in determining the significance of the various possible means of transmission is illustrated by the report of Schoenleber (1940). Starting with the conviction that food-handlers were disseminating the infection in an industrial installation in the tropics, Schoenleber treated food-handlers and the general population, and enforced sanitary regulations. In the process the amebiasis rate dropped and the elimination of the infected food-handlers was cited as the cause.

I do not want to give the erroneous impression that I, in any way, doubt that water, flies, or infected food-handlers can transmit amebiasis. However, there is often a marked difference between what can occur and what does occur. In endemic areas the opportunity of direct fecal contamination makes it difficult to determine the exact vehicle which conveys the organism to the mouth but suggests to me that these oft-cited specific means actually play comparatively minor roles.

It may be noted that the cysts of *E. histolytica* are quickly killed by drying, but I will remind you that their survival in water is comparatively short unless the water is very cold. Furthermore, the cysts settle out of water quite rapidly unless the water is in movement or otherwise agitated. Perhaps these vehicles are utilized in areas of low endemicity, but I will remind you that gross carelessness in fecal disposal is not the exclusive prerogative of the South. It occurs in cities such as New York, Chicago, Baltimore, and Philadelphia, though to a much lesser extent than in certain of the rural areas in the South.

In this connection, it may be well to reflect a moment on the quantitative aspects of the infection. Although Beaver *et al* (1950) succeeded in infecting human volunteers with 2000 freshly isolated cysts, none developed evidence of disease. Visualize the activity of a fly, or even a swarm of flies, carrying such an infective dose even with the ample source of material described above. It would require conscious effort even for an infected food-handler to supply such infective doses. It is, of course, possible that a single cyst could produce the infection, but I will hazard the guess that it would not be a common occurrence. Tremendous doses are needed to infect experimental animals. Sadun *et al* (1950) reported that not all guinea pigs became infected even with 5 million cysts. Admittedly, the guinea pig is not a normal host for this parasite and no direct numerical comparisons on the minimum effective dose are permissible. However, Walker and Sellards (1913) were not as successful as Beaver and his co-workers in attempts to infect human volunteers. With one inoculum they were able to infect only 17 of 20 men and only one more became infected after two additional feedings. Of the 18 who became infected, only four developed disease. The relation between continued exposure and increased attack rate in the Chicago hotels in 1953 was mentioned earlier.

In short, we have a picture of massive exposure with massive doses or continued exposure with the same result associated with a high infection rate and a high disease attack rate. I suggest that even a superficial study of scatology reveals that there is at least a rough correlation between increasing degrees of carelessness in fecal disposal and increasing prevalence rates of amebiasis, that highly endemic areas are areas of promiscuous defecation, and that this more than compensates for the parasite's lack of resistance to tropical and subtropical climates. I further suggest that food-handlers, flies, nightsoil-fertilized vegetables, and (except where we contrive to grossly contaminate our modern facilities) basic water supplies play comparatively minor roles in the maintenance of highly endemic areas or the development of serious epidemics. Our acceptance of these without adequate demonstration are merely easy explanations, miss the fundamental problem, and approach the worship of "sacred cows". I fully realize that this is parasitological heresy, but if such heresy, by chance, stimulates the gathering of more adequate data, I will be more than satisfied. To the various other factors, such as the immunological status, the nutritional status, and concurrent disease which must be considered for a complete understanding of the ecology of this parasite, Eyles, Jones, and Smith (1953) have added the question of racial differences in susceptibility.

LEUCOCYTOZOON

Malariology has had a long and often controversial history. The ecological aspect of the parasites, mosquito vectors, and vertebrate hosts have been extensively reviewed several times so that I will not consume your time here in an inadequate coverage. However, several related forms offer some wonderful opportunities for the ecologically minded experimental biologist. Both *Leucocytozoon* and *Haemoproteus* offer excellent opportunities. The former will serve as an illustration. The evidence that schizogony is restricted to the internal organs and that the gametocytes alone appear in the peripheral blood sets these forms apart biologically and taxonomically from *Plasmodium*. And yet, I wonder whether or not such forms as *Plasmodium gallinaceum* and *P. falciparum* may represent biological links between the Haemoproteidae and such forms as *Plasmodium vivax*. You will recall that Lewert (1950) presented experimental evidence that the gametocytes of *P. gallinaceum* were derived primarily, if not exclusively, from schizonts in the tissues. Likewise, I have seen relapsing *P. falciparum* infections in which the first parasites in the circulating blood were exclusively gametocytes and only later were trophozoites seen. There is some similarity between these and relapsing *Leucocytozoon simondi* infections in ducks. There is much yet to be learned about *Leucocytozoon*. The unqualified evidence by Huff (1942) from bone marrow preparations that *L. simondi* gametocytes develop exclusively in the white cell series is in direct conflict with the equally unqualified evidence by Cook (1954) that round gametocytes in the circulating blood, at least, develop exclusively in the red cell series and primarily in reticulocytes. Are there two species involved? Possible support for this is seen in the evidence that both the round and elongate gametocytes undergo maturation (Fallis, Davies, and Vickers, 1951; Rawley, 1953). The round gametocytes have been considered as immature and the elongate forms as mature in much of the literature. However, none of the workers cited nor Chernin (1952b) were able to find any evidence of a transition

between the round and elongate gametocytes. There are difficulties about the two-species concept, an important one being that both round and elongate gametocytes occur in almost every host. It is possible that they result from schizonts in different tissues, viz., Huff's hepatic schizonts and megaloschizonts (Huff, 1942; Cowan, 1955; Fallis, Anderson, and Bennett, 1956; and Newberne, 1957).

The gametocyte pattern in the circulating blood does not clearly resolve the question. Chernin (1952b) found that gametocytemia occurred within a week after exposure, rose rapidly to maximum and subsided to scarcely detectable levels within two to three weeks. More often than not the earliest gametocytes seen were round, or at least the round form predominated, followed by an increase in the elongate gametocytes. Exceptions, however, occurred—occasionally gametocytemia was initiated by preponderantly elongate forms. There was rarely any numerical correlation between the number of round and the succeeding number of elongate forms. The sequence in one bird with an interrupted parasitemia gives the sharpest indication that maturation did not take place in the bood. Round gametocytes were exclusively found on the onset of parasitemia. This condition continued for 10 days followed by the complete absence of parasites for two days and then a recrudescence with exclusively elongate forms. Throughout the fall and winter, when gametocytemia was at barely detectable levels, about 20% of the gametocytes found were round and the remaining 80% elongate. In the spring relapse the gametocytes were predominately, and sometimes exclusively, the elongate forms. The biological succession and the ecological relations of these two types of gametocytes and the underlying tissue stage in the duck remain to be unraveled.

The further elucidation of the relapse phenomenon offers a fascinating area for investigation. Chernin (1952a) found that relapse was closely associated with the onset of egg-laying in the early spring. When the onset of egg-laying was advanced a month to six weeks into late winter by increasing the daylight artificially (to 16 hours), the gametocytemia relapse was similarly advanced. When the onset of egg-laying was delayed by maintaining short days (5 hours) and long nights well into the late spring, the gametocyte relapse was delayed by the same ratio. Chernin attempted to induce gametocyte relapse with certain female hormones without success. No one, least of all Chernin himself, would contend that this aspect of the subject has been exhausted. Further studies may prove fruitful. However, the female hormones are not alone involved because the male ducks also relapsed at the time they became sexually active. It would appear more profitable to consider some of the more basic physiological changes associated with the longer daylight, changes which in themselves may precede and stimulate sexual activity and perhaps independently the parasite's activity. Whatever the mechanism, the process of its elucidation would be fascinating and the results satisfying.

The evidence that black flies are involved in the transmission of *Leucocytozoon* is over a quarter of a century old but not until the work of Fallis, Davies, and Vickers (1951) and Fallis, Anderson, and Bennett (1956) were any accurate details available. More details need to be filled in. The relative susceptibility of different strains and species are needed to clarify the epizootiology. The ecological adjustment of the parasite in the fly to different climatic conditions has a direct

bearing on the epizootiology. Why, for instance, should transmission in Northern Michigan be delayed until late June or early July and terminate shortly after the middle of August with heavy transmission largely restricted to the month of July (Chernin, 1952), whereas farther North the period of transmission is much longer? Fallis and his coworkers in Algonquin Park in Ontario found very active transmission beginning in May. Are different strains or species of flies involved in the two areas, are there different duck populations with resulting different times in the gametocyte relapse, or are there different local ecological conditions which directly or indirectly influence one or more of the above? Perhaps, it is simpler to assume that these epizootiological differences result from differences in the ecology of the black fly vectors, perhaps even different species of black flies. Clarification is needed. Incidentally, many details are still missing on the development in the black fly despite the pioneer work of Fallis and his colleagues in Ontario. From the available information, I have a suspicion that biologically *Leucocytozoon* may have, taxonomically and biologically, more than a mere generic difference from *Haemoproteus*.

FILARIASIS

That different epidemiological patterns do occur as a result of ecological differences in the vector as well as biological differences in the species of parasites are too well known in malaria to warrant discussion here. However, these differences have not been as fully appreciated in the ecology of some other insect-borne infections. The manner in which differences in the ecology of the parasite, the vertebrate host, and the vector combine to produce basic differences in the epidemiology of the disease is illustrated by the species of *Wuchereria*. In most areas of the world in which *W. bancrofti* is endemic, the microfilariae are found in the peripheral blood predominately at night, and transmission is primarily by night-biting highly domestic anthropophilous mosquitoes. Under such circumstances, human infection is acquired early in infancy and childhood with maximum microfilaremia rate occuring by the age of 10, and it may remain relatively constant thereafter. Not infrequently, however, there may be a reduction in the microfilaremia rate in the older age groups.

Quite a different ecological and epidemiological situation exists in a group of islands in the South Pacific. There the microfilariae lack any evidence of a nocturnal periodicity and may, in fact, have a slight diurnal periodicity in the peripheral circulation (Eyles, Hunter, and Warren, 1947). Transmission is by mosquitoes with a short flight range, scarcely as much as 100 yards, which are confined to densely vegetated areas (Jachowski, 1954). Sylvan transmission is the rule. Under these conditions, infants are rarely infected and the rate of microfilaremia rises slowly through childhood. There is commonly a sex difference with adolescent and young adult males in their work and travels in the jungle and plantation area acquiring the infection more quickly than the less traveled females (Jachowski and Otto, 1952, 1955). Filariasis may be classed as an occupational hazard. These several facts were all known before the exposure of American troops to this infection in Samoa during World War II (Bahr, 1912; Stephens and Yorke, 1921; O'Connor, 1923; Buxton and Hopkins, 1927; Buxton, 1928) and confirmed by researches during the war (Byrd, St. Amant, and Bromberg, 1945). However, the full ecological and epidermiological implications were

not appreciated, apparently because each facet of information was separately considered rather than being fitted with the full available information into an ecological pattern. Unfortunately, as with malaria and periodic filariasis, control was centered in the barracks area and focused on a night hazard. Furthermore, the native villages were off limits at night. Actually, the infection was acquired in the jungle areas in the daytime and was completely unaffected by the control procedure.

It is interesting to speculate on the origin of the non-periodic filariasis. We see this as a mass phenomenon in one type of ecology in a limited number of South Pacific Islands on which periodic filariasis is unknown. Here the dominant mosquito is, or has been until recently, the daytime feeding *Aedes polynesiensis* or one of its close relatives. Continuous transmission has resulted in selecting a strain of filaria which has largely lost its capacity to develop in *Culex quinquefasciatus*, the night biting mosquito which is probably the single most important vector of periodic *W. bancrofti* (Rosen, 1955). But does this signify that non-periodic filariasis is confined to such areas? Is it not possible that non-periodic strains or mutants occur as numerically minor variants in areas dominated by strains with a nocturnal periodicity? One can visualize that such non-periodic variants would enjoy only limited success competing with the periodic strains when the total ecology favors the latter. If this is true, it is probable that, if sufficient comparative night and day bloods are examined, occasionally non-periodic infections will be discovered. Despite the fact that relatively few comparative night and day bloods have been examined, at least three non-periodic infections have been discovered in the "periodic areas", one each in the Philippines (Ashburn and Craig, 1907), in Africa (Pinto and de Almeida, 1947), and Cochin China (Canet, 1950). The corollary is that from such variants an endemic area of non-periodic filariasis will develop in a favorable ecological environment which must include, of course, the preponderance of daytime feeding vectors.

Wuchereria malayi offers some interesting ecological variations. The microfilariae have a nocturnal periodicity, but it is much less pronounced than is the nocturnal periodicity of *W. bancrofti*. Transmission is by night biting mosquitoes which readily enter human habitations to feed. However, the larvae of the vector mosquitoes (*Mansonia*) are dependent upon certain water plants (*Pistia*) to which they attach. In some areas of Malaya they are found only in the jungle and plantation areas some distance removed from the villages. Poynton and Hodgkin (1938) found evidence here, as in Samoa, that there was an occupational hazard but with a somewhat different pattern. Here men, lingering at the plantations or enroute through jungle during the twilight hours, appear to provide both the source of infection for the mosquito vectors and the recipients of the infection from the mosquitoes. Nevertheless in this same area according to Jachowski (1955), Edeson and Wilson both found that, as with periodic *W. bancrofti* infection, the microfilaremia rate rose rapidly to reach a peak within the first decade of life. Thereafter, the microfilaremia rate dropped off in both sexes, but it continued at higher level in males than in females through adolescence and early adulthood. Attempts to elucidate further the ecology and resulting epidemiology is complicated by the coexistence of *W. bancrofti* (periodic) and a filaria in the Kra monkey morphologically indistinguishable from *W. malayi*, which may or may not be infectious for man.

Canine filariasis has also posed some troublesome ecological problems in United States. The presence of a high prevalence rate of microfilaremia in the absence of adult worms in the heart in marginal areas has caused considerable confusion (Mundhenk and Green, 1939). The recurrent evidence of the larval development of a canine filaria in the flea (Brown and Sheldon, 1943; Summer, 1943; Stueben, 1954) is undeniable but *per se* created new problems without resolving any. The recent finding of a species of *Dipetalonema* in dogs in association with development in the flea is fundamental to the resolution of a number of problems (Newton and Wright, 1956, 1957). The indications from blood examinations that canine filariasis is more common in the Middle West than formerly appreciated, is contrary to the available evidence on the biology and ecology of *Dirofilaria immitis* in the mosquito vector (Otto, 1949). The flea, during its sojourn on the dog, would offer a more favorable temperature gradient to the developing larvae than would the free-flying mosquito in northern communities. Thus, it is reasonable to believe that much of the canine filariasis reported from these northern areas is actually *Dipetalonema* rather than the heartworm, *D. immitis*. However, the matter may not be quite so simple. Increasing numbers of dogs in northern areas are being found, on necropsy, heavily burdened with adult *D. immitis*. Many of these have unquestionably acquired their infections in the South, but some are alleged to be local dogs which have never been out of the local northern area in which they are found infected. Obviously, more data are needed on the differential distribution of *D. immitis* and *Dipetalonema* sp., as well as any hitherto unrecognized species. Probably, an evaluation of the ecology of the two forms, particularly with reference to the temperature requirements and tolerances, respectively in the mosquito and the flea would be helpful. Schnelle and Young (1944) found periodicity to be a common but not consistent phenomenon in canine filariasis. Perhaps the discrepancies are species-related. Delineation of the differential pathology, clinical manifestations, and response to therapy is desirable. Here we have a very precise example of conflicting, often irreconcilable, ecological data which could not be rationalized until there was taxonomic clarification. With the new taxonomic evidence, the ecological problems offer new challenges to the investigator. Any clarification would be a welcome contribution to the harassed veterinary practitioner.

Although a 100% prevalence rate with canine filariasis may be closely approached in such highly endemic areas as Florida, I know of no area in which the prevalence rate with *W. malayi* or *W. bancrofti* (periodic or non-periodic) is appreciably more than half this rate in any age group despite a continuing exposure to infection. In part, at least, this appears to be host pressure on the parasite. Pressure which has developed as the result of long repeated exposure to infection, i.e. the development of immunity that adversely affects the parasite and which, in part at least, may be equally unfavorable to the human host. Although bacterial complications undoubtedly play a part in the development of elephantiasis, it appears to me probable that this condition results primarily from the defensive mechanism of the body against recurring crops of worms. I suspect that the tissue reaction is often restimulated in the sensitized host by the inoculation by the mosquito of infective larvae which are never able to complete development and which may never even reach the location of the actual elephantiasis. Wilson and Reid (1951) found that among 102 cases of elephantiasis in Malaya only seven

had a demonstrable microfilaremia, whereas they found 40% showing microfilaremia among over 1300 asymptomatic individuals. Poynton and Hodgkin (1938) found microfilaremia (5%) in elephantoid cases only in those with slight elephantiasis. Among those classified as moderate or severe no microfilariae were demonstrated. With *W. bancrofti* infections elephantiasis of the genitalia and the legs is more common than elephantiasis of the arms. In women the mammary glands may be involved. When *W. malayi* was first discovered, it was said to be most commonly associated with elephantiasis of arms. More recent evidence, summarized by Jachowski (1955), indicates that the elephantoid condition with this species is characteristically confined to the legs both in males and females. Only rarely are the upper limbs or the genitalia involved. The nosogeographic distribution of the elephantoid tissue and relation to resistance are both reflections of the ecology of the parasite in the primary host, but is elephantiasis the most characteristic clinical feature of filariasis?

An educational institution comes to be known by its most advertised curriculum and an industry by its most advertised product. In much the same manner, a parasite comes to be associated primarily or even exclusively with its most dramatic effect upon the host, its most advertised effect. To the casual student this may give an entirely erroneous picture, and it may result in considerable confusion and frustration to the more careful observer as he discovers how rarely the "average" or "typical" course of the disease develops with a given infection. How well I recall my search for the grotesque elephantiasis, the pictures of which are used to illustrate accounts of filariasis in almost every textbook of parasitology or tropical medicine and in a number of, shall I say, popular accounts of this infection. Advanced elephantiasis is rare in the Caribbean Islands, even on those islands where the infection is common. I was told the condition was common in the South Pacific Islands. However, on Samoa it was very rare—go to Tahiti or Fiji, I was told. I missed Tahiti, but on Fiji again the condition was rare. I was there assured that it was plentiful on the Gilbert and Ellice Islands. Jachowski (1955) reports elephantiasis in 10% of the adult males and 5% of the adult females in Malaya. But, I haven't completed the circuit—my survey is not complete. Perhaps the large grotesque elephantoid scrota and limbs are more common on some islands or in some communities. I do not know. But I have learned that there are many areas with a high prevalence rate of infection but with a comparatively low rate of the grotesque elephantiasis. It is common enough and it can never be casually considered. However, in many areas particularly in the South Pacific the debilitating and excruciating pain of recurrent attacks of filarial fever is far more prevalent and occurs both in the elephantoid cases and in those who show no signs of permanent elephantiasis. In my experience in the South Pacific, these recurrent attacks of filarial fever with its transient lymphangitis and lymph adenitis ("mumu") more nearly characterize the disease and are far more debilitating to the total population than the more widely advertised growths. These attacks of filarial fever hold a clue to the host response to the infection.

HOOKWORM

And yet, the above experience was not new to me. Many years before, disillusion traumatized my tender years when I discovered how rarely "typical hookworm disease" was encountered among those harboring hookworm infection in south-

eastern United States. Studies on the ecology of the free-living stages of the hookworm and on the ecology of the human host by Cort and his students and colleagues made it all too evident that there were other aspects to this ecological picture. The evidence garnered in the experimental laboratory has subsequently shown that both man and dog have the capacity to develop a powerful and highly protective immunity (Cort and Otto, 1940; Otto, 1948). It has also become evident that general malnutrition or specific deficiency states can and do reduce the effectiveness of the immune response. It is suggested, but not demonstrated, that concurrent infection may similarly reduce the protective immunity. Nevertheless, the mechanism of immunity is essentially as in antibacterial or antiviral immunity. Among other things Thorson (1953) has identified in the closely related *Nippostrongylus muris* a lipolytic enzyme as the antigen which stimulates the protective immunity and more recently (Thorson, 1956) has shown that immune serum will neutralize the proteolytic enzyme of hookworms. Kerr (1936) has shown that the retarded worms in the tissues of immunized animals are engulfed in the inflammatory response characteristically seen in immunity to bacterial infections. And yet, we have not directly harnessed the information into practical utilization. Perhaps, such practical utilization will be possible when the antigen can be synthesized in the laboratory in quantity. And yet I wonder. Can we assume that the capacity for biological response to attack is restricted to the host? Drudge, Leland, and Wyant (1957) have found the nematodes (*Haemonchus contortus*) have developed, apparently by natural selection, a drug-resistant strain just as bacteria and protozoa have developed drug-fast strains. Can we assume that the hookworms cannot develop, or indeed already have not developed, a response to the host immunity? In the immunized host, a few hookworms persist. Is this, as many of us have assumed, due to a reduction in the immune level with reduced antigenic stimulus, or are other factors involved? Certainly, we have a situation in which a continuing low level of infection exists in sizable populations without specific clinical signs of illness. Only when a combination of ecological circumstances combine to produce a more favorable opportunity for the worms do we find "typical" hookworm disease. Acute hemorrhagic disease produced exclusively or primarily by massive invasion of hookworms is even more rare. The classical example is that reported by Ashford, Payne, and Payne (1933) from Puerto Rico. In the United States both acute hemorrhagic hookworm disease and the more "typical" chronic debilitating disease is much less frequently seen than when the late Charles Wardell Stiles began his militant campaigning a half century ago. And from what has this reduction resulted? I suspect that essentially the same "bonification", which the Italians invoked and the League of Nations adopted over a quarter of a century ago to explain the malaria recession in Europe, has been primarily involved. This would include economic improvement which would directly provide the opportunity for improved nutritional status, improved housing, and increased use of shoes. Continuous educational pressure from health agencies and increased contacts with gasoline filling stations with their inevitable rest rooms have undoubtedly extended Stiles's "privy consciousness" into the lower economic groups. A half a century of intermittent therapy by public agencies has probably also contributed. It would appear to me that in the aggregate these and related factors have combined to produce unfavorable ecological environments for hook-

worm, both within and without the human host, in much of southeastern United States.

Neverthless, subclinical, and some clinical, infections persist. It is in keeping with the current world movement on improved health to inquire into the health significance of these subclinical infections. The rapidly accumulating evidence of reduced weight gain and unfavorable food conversion due to subclinical worm infections in livestock (Boughton, 1957) suggests that the question, at least, warrants investigation in man. Are we ready to consider low level anthelmintics in our processed food in somewhat the same manner as in livestock feed? Should we consider adding anthelmintics to the universally used table salt as has been done in salt licks for cattle and as antimalarials have been added experimentally in table salt? Perhaps there are other ways to completely disrupt the requirements of the hookworms of man. However, the ecology of both man and parasite should be considered.

To support Philips' evidence that there is always "something new under the parasitological sun", it seems appropriate to note in passing that several workers in the last decade have used hookworms therapeutically to reduce the pathologically high erythrocyte levels in polycythemia vera (see Myhre and Wallace, 1956).

ECHINOCOCCOSIS

Recent studies, with which I have been associated, involved some serious serological, epidemiological, and clinical conundrums due to the too easy assumption that the ecology of *Echinococcus granulosus* encompassed the ecological relationship between man and worms of this genus. Not until the careful morphological work of Rausch and his co-workers in Alaska (Rausch, 1952, 1953; Rausch and Schiller, 1956; Rausch and Yamashita, 1956); and of Vogel (1955) in Germany revealed a species distinct from *E. granulosus* did we appreciate that new concepts were in order. The careful ecological and epizootiological studies by Rausch and of Vogel have given us a base line. In contrast to *E. granulosus*, which utilizes dogs and wolves as the definitive host and ungulates as the intermediate host, *E. multilocularis* utilizes dogs and foxes as the definitive host and rodents as the intermediate host. Many of the ecological relations to man have yet to be worked out, such as man's relative susceptibility to the two species and the antigenic and serological response in man to the two species. Again, as with amebiasis and canine filariasis, the ecological relationship has been confused by the lack of finite taxonomic information.

TRICHINOSIS

An entirely different type of ecological problem is involved currently in our attempts to develop additional preventive measures against trichinosis. One point troubles me a great deal. This has to do with the much publicized proposal to use gamma rays or a similar procedure for the destruction of *Trichinella* larvae in pork. Such a procedure, no matter how efficient in operation, could be applied basically only in the larger abattoirs. The only outbreaks of trichinosis, which I have known, involving more than a single individual have resulted from home-butchered hogs or from those butchered in small local abattoirs. I wonder, then, if most of the radiant energy wouldn't be used on the trichinosis-free hogs and the minor sources

of infection, leaving the present ecological adjustment between man and *Trichinella* essentially unmolested for posterity. Why not capitalize on the current concern among swine raisers over the transmission of virus infections from garbage to swine. The ecology of *Trichinella*, virus, swine, and man have a curious and highly suggestive point of common impingement. Could we not use this knowledge for the benefit of both man's health and his food supply (pork) more effectively than by the wholesale treatment of pork to destroy the worms? The question, I think, is worth consideration.

CONCLUDING STATEMENT

In these few examples, I have not concerned myself so much with established fact as with certain areas which I believe are even today in need of further clarification. To know the parasite completely we must know more than its taxonomy, morphology, and life cycle, fundamental as these are. To know the parasite completely, we must know its metabolism, the enzyme systems it uses, its environmental requirements, and all other aspects of its physiology. No one parasite is completely known, but in many cases the many facets of information available give us a clue at least to its ecology. It is human nature to respond favorably to finite data but to be wary of vague subtleties. Thus, when two sets of data appear to be in direct conflict, there is an almost automatic reaction directed towards learning which is the truth and which is in error. While these extremes do occur and must be resolved, more often than not this appearance of conflicting information signifies that there are large gaps in our knowledge. These gaps in our knowledge of the ecology of the parasites are wider and more frequent than any other aspect of parasites and parasitism. We have not always readily utilized or even appreciated some of the information available. Malariology serves as an outstanding example of how many disciplines can contribute to our knowledge of the ecology of a parasite, including the ecology of its hosts. Before the advent of the residual spray simplified our task, the ecological information was being effectively utilized in control measures. It was largely because of this background that the concept of residual spraying was put to use so promptly and effectively. A full appreciation of this will reveal that in many other cases we do not have comparable information on the ecology of the parasite concerned. Schistosomiasis, which has succeeded malaria as the Number One disease, holds many challenges.

In some cases we have not fully utilized or appreciated the information available because we have looked too long and too steadfastly at a few data to the neglect of other apparently conflicting, and thus "erroneous data". In some cases statements, which were originally presented as theories, speculative interpretations, or working hypotheses, have been repeated so often that they have unfortunately come to be accepted as demonstrated fact. An understanding of the ecology of a parasite and the resulting parasitism requires an appreciation of the many facets, including the ecology of the host and how parasite and host influence each other. This requires further exploration of the controversial areas, and in some cases this requires looking critically at currently accepted concepts and subjecting them to further evaluation. The future offers opportunities unlimited to the ecologically minded parasitologist.

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RESEARCH NOTE

A SARCOPHAGID LARVA (DIPTERA) FROM THE TERMINAL AMPOULE OF THE PINK SHRIMP, *PENAEUS DUORARUM* BURKENROAD

In connection with routine examinations of *Penaeus duorarum* for parasites a Diptera larva was found in a spermatophore located in the terminal ampoule of a male specimen collected from Tampa Bay, Florida. We are indebted to Curtis W. Sabrosky, Insect Identification and Parasite Introduction Section, Entomology Research Division, U. S. Department of Agriculture, for identifying this specimen as a very early second-instar Sarcophagidae larva.

Sarcophagid larvae consume flesh, dead organic material or live in insects. They have been reported from man as well as other animals. However, the present account appears to be the first report of the invasion of the commercial pink shrimp, *Penaeus duorarum*, by a sarcophagid larva.

During the evening of May 5, 1957, at approximately 8:00 P.M., Mr. Kenneth D. Woodburn, Biologist, Florida State Board of Conservation Marine Laboratory, completed a regularly scheduled shrimp haul with a small frame trawl. The contents of the trawl, including fish, shrimp, other invertebrates, grasses, algae, etc, were placed in a large bucket. The bucket was covered immediately and returned to the laboratory where it was stored at approximately 40° F. Approximately 1 hour elapsed from the time the haul was made until it was placed under refrigeration. The following morning, shortly before 10:00 A.M., while examining the terminal ampoule of a male specimen of *P. duorarum*, unusual movements were noted by one of us (B.E.) within a spermatophore, or sperm-bearing sac. When the spermatophore was broken the living parasite (Fig. 1) was found. The parasite measured 2.3 mm in length and approximately 0.5 mm in width. It was extremely active when removed from the terminal ampoule. There was no anatomical evidence indicating the path by which the larva entered this organ. No attempt was made to rear the larva.—ROBERT F. HUTTON AND BONNIE ELDRED, *Florida State Board of Conservation Marine Laboratory, St. Petersburg, Florida.*

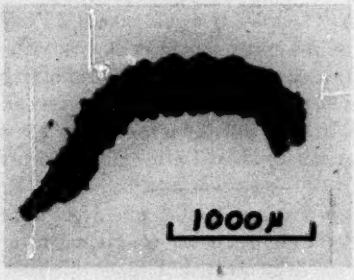

FIGURE 1. Sarcophagid larva from *Penaeus duorarum* Burkenroad.

AN APPROACH TOWARD A COURSE IN THE PRINCIPLES OF PARASITISM[1]

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FOREWORD

In an age when scientific discovery proceeds with such rapidity as at present it is not easy to keep teaching (even at the undergraduate college level) in pace with knowledge—to conserve the firm essentials of older learning, add the new, show the unexplored frontiers, balance topic against topic, meet changing student needs, yet keep the whole of any course or textbook within workable limits of time and space.

The solution in every case devolves upon the individual teacher within the context of a particular college or university and of a specific student group. However, courses in a subject like parasitism or parasitology must often be given by biologists who are not primarily experts in that field. Even those who are specialists may encounter difficulty in keeping up with all facets of the field and the many other biological areas that impinge upon it. It seems reasonable to propose that collaborative effort to examine course offerings and to suggest new possibilities may be provocative and productive, may aid individual enterprise, and may spur advances in teaching in the same way that conferences and symposia may promote the progress of research.

This hypothesis led the Subcommittee on Instructional Materials and Publications of the Committee on Educational Policies, Division of Biology and Agriculture, National Academy of Sciences-National Research Council, to propose a tactic for exploring new approaches to content and organization of undergraduate courses in the biological sciences. The idea adapts the research conference technique to thinking about courses. If informed biologists in a given field feel that courses in that field often tend to follow somewhat outmoded stereotypes, that teaching in a subject needs critical scrutiny, an *ad hoc* panel could be set up to suggest guide lines as to content and organization. This panel would consist of biologists representing different branches of the discipline who would review the whole design of the course, considering its functions, what understanding and information students who might take the course need, and how it can be related to the rest of a biological curriculum. Applied and professional aspects should be taken into account, but many biologists urge that undergraduate courses should primarily emphasize basic principles, thus contributing to the student's maturation as a biologist. Having defined objectives and reviewed the field as it now stands, the panel would propose topics and suggest their relations, noting, for example, what traditional material might give way to newer developments, what sequence might most effectively depict the field as a body of coherent knowledge, and, particularly, how to show it as an area for con-

[1] Report of the *ad hoc* Panel on Parasitism Courses, Committee on Educational Policies, Division of Biology and Agriculture, National Academy of Sciences—National Research Council. The Panel members wish to express their gratitude for the assistance rendered by Richard E. Paulson, executive secretary of the Committee on Educational Policies.

tinuing investigation. The panel would publish a single report. Its ideas and recommendations would thereby be made available for such use as teachers and textbook writers care to make of them. The report would be fully open to criticism; one of its main functions would be to stimulate thinking and debate about courses in the given field, out of which better ideas than those the panel proposes might well emerge.

Such panels, the Committee believes, do not threaten to impose any specific patterns upon teaching. On the contrary, their role is to question current conventions, looking at the field afresh, suggesting new approaches for consideration by individual teachers. Panels could be organized by any interested group, either on a completely informal basis or under the sponsorship of an appropriate professional society or scientific organization.

To test the idea, the Committee on Educational Policies, aided by a grant from the National Science Foundation, set up two experimental panels, one on Parasitism Courses and one on Systematic Botany Courses. Biologists in both fields have been keenly interested in the project, and professional societies have generously suggested candidates for the panels. This is the report of the Panel on Parasitism Courses. Appointed in the fall of 1956, the Panel met first on December 3–4, 1956, at the National Academy of Sciences in Washington, D. C., and again at the University of Michigan on June 3–5, 1957.*

The Panel visualizes a basic course in the principles of parasitism with particular emphasis on animals, available at the junior level, and open to students who have had at least 1 year of chemistry and 1 year of biological sciences. Two years of biology would provide better background and 2 years of chemistry still better, but the Panel believes that the course should be open to as many potential biologists as possible. The course emerges from traditional animal parasitology, but is designed to supply basic background for students interested in zoology, animal ecology, microbiology, parasitology, medicine, veterinary medicine, wildlife technology; properly organized, it could also serve students in botany, and plant pathology.

The major problem, the Panel felt, was to attempt to lay out these basic principles, as this group sees them. This is done in Section 2, Précis of Parasitism. The Précis defines content. How it is organized in a course or text depends upon the individual teacher or author. There is no single "right" way to teach anything. However, we think there are some basic ideas which instructors will want to consider in designing a course. These are discussed in Section III, and Section IV gives three examples of course outlines that attempt to present the principles of parasitism.

Our work will have been thoroughly worthwhile if this report leads to the development of new courses and stimulates serious and continuing review of teaching by all who present basic courses in parasitism or parasitology.

PRÉCIS OF PARASITISM

I. Introduction

A major branch of biology, *ecology*, is concerned with interrelations of organisms and environments, both physical and biotic. One area of ecology deals with systems of biotic relations, systems in which one organism lives on or in another in

* Dr. James Dickson of the Department of Plant Pathology at the University of Wisconsin participated in this meeting as alternate for Dr. A. J. Riker. Dr. Dickson also read and contributed to the final report of the Panel.

order to obtain sustenance: One or both organisms may derive benefit from the association; one or neither may be harmed. These are *symbiotic* relationships, of which 3 types are known: 1) *mutualism*, in which both members benefit (*e.g.*, lichens, symbiotic bacteria, possibly so-called parasitic males and parasitic embryos); 2) *commensalism*, in which one benefits and the other is not harmed (*e.g.*, inquilines); 3) *parasitism*, in which one benefits, the other is harmed. The last is the area of biology with which this report is primarily concerned.

Because of its importance for medicine, agriculture, conservation, and the biology of all kinds of organisms which may harbor parasites, parasitism is a relatively well known branch of ecology. In particular, more progress has been made in analyzing experimentally the intimate mechanisms of association than is the case for most examples of other ecological systems. Parasitism thus claims attention both in its own right and for the light it casts upon ecology as a whole.

Broadly, symbiosis, including parasitism, can be related to the phenomena of *antibiosis* and to heterotrophic nutrition. A continuum of relations between food and feeder can be found, ranging from clear predation through ambiguous types like the alimentation of weasels and blood-sucking mosquitoes, then to parasitoids and hyperparasitoids like chalcid wasps, and finally to clear-cut symbiotes and parasites.

Of special interest to general ecology, evolution, and the biology of the organisms involved is the role of *adaptation* in symbiosis and parasitism, both the specific adaptations of parasites to specific environments and the adaptations and reactions of the host to an environment changed by introduction of the parasite.

Although there is a clear trend to a biology of symbiosis, which would include parasitism, so that the basic course in the subject may eventually be one in symbiosis, present practice seems to call for a course in parasitism. For the present it seems useful to follow this practice, while continually bringing the wider range of relations into the picture.

Parasitism is a biological phenomenon, study of which is now subdivided among plant pathology, animal and human pathology, parasitology (the branch of zoology concerned with animals with a parasitic mode of life), economic entomology, mycology, bacteriology, virology, and immunology. Both plant pathologists and animal parasitologists may further be subdivided according to the groups of parasites on which they work: arthropods, helminths, fungi, bacteria, viruses, etc. Nevertheless, it is becoming clear that there will eventually emerge a common body of basic principles which underlies all of these specialties. This suggests that the first course basic to all of these fields may one day be a course in parasitism or symbiosis. Such a course would also provide all varieties of biologists with an introduction to this important area of ecology. Hence, while this précis is conceived primarily in terms of animal parasitology, the wider applicability of principles should continually be considered, and the zoologist should not hesitate to draw principles or examples from plant pathology where knowledge provides concepts or examples not yet developed for animal forms. The complementary genetics of host-parasite systems, for example, is far better understood for certain fungal parasites of grains than for any animals.

Certain basic definitions seem to be essential. *Parasitism* is a type of symbiotic association in which one organism, the parasite, lives on or in another organism, the *host*, and draws its sustenance from it, with benefit to the parasite and harm

to the host. Parasites are thus pathogens, but the association need not involve such severe damage as to produce significant disease. Tolerance to parasitic infections has selective advantage to the individual host, and there are many examples of series of hosts that differ in the magnitude of the effect of the same level of infection. The parasite may subsist upon a social group as a *supraparasite* (*e.g.*, termitophiles), may live on an organism as an *ectoparasite* (*e.g.*, ticks), or may live in its host as an *endoparasite* (*e.g.*, cestodes). Endoparasites may live within body spaces such as the gut, in true body cavities, in blood vessels, in tissues, or in cells. The degree of dependence of parasite upon host ranges from *obligate* parasitism, in which the parasite cannot live otherwise, to *facultative* parasitism, in which the parasite can exist either as a parasite or as a free-living organism; here parasitism intergrades with the scavenger, saprozoic and saprophytic modes of life, in which the dependent species requires nutrients from other organisms but can obtain them from dead organisms or detritus resulting from decay. Parasitic associations have apparently arisen independently many times in the course of evolution. Parasites are represented in most of the plant and animal phyla, and occur in or on practically all living organisms.

Parasitism has been known by man from antiquity and helped to foster the notion of spontaneous generation until the 18th and 19th centuries, when there was convincing proof of the doctrine of biogenesis with the demonstration of its application to microorganisms. During the 19th century the so-called germ theory of disease—the concept that infectious disease is the result of the invasion of the host by specific pathogenic parasites—was firmly established. Parasitology and other sciences concerned with parasitism then focused on the identification and description of parasites, their host ranges, and the avenues of transmission from host to host, which often turned out to involve complex life cycles. Neither the taxonomic nor the descriptive phase of parasitological investigation has yet been exhausted, but during recent decades interest has increasingly turned to mechanisms by which hosts defend themselves against parasites and develop immunity, and to studies of the intimate physiology, biochemistry, and genetics of parasitism.

II. Properties of Parasites

In any consideration of parasitism as a phenomenon it is not possible to isolate completely the properties of either member of the association. The most essential characteristic of the association of host and parasite lies in the interaction of the two. However, for practical purposes in the following discussion, the attributes of the parasitic member which contribute to its success in the association can be isolated.

Parasites (and symbiotes) differ from non-parasites in living in direct association with the body of another organism. The host body is a *habitat* having physicochemical properties allowing the parasite to satisfy its life needs for survival and reproduction. Having independent evolutionary origins, parasites belonging to different taxonomic groups seem to have little in common with each other except their dependence on the host.

A. *Adaptations for reaching a host.* Successful parasitism may be thought of as a continuous series of events, the successive steps being: entrance, establishment, emergence, and transmission (Smith, *Science* **54**: 99–108. 1921). In addition to these needs, the parasite must have adaptations for nutrition and reproduction.

Parasites may reach the host by chance or through specific behavioral activities. The latter are based on the irritability of protoplasm and the presence of coordinating systems in the various phyla of organisms in which parasitic species are found. These coordinating systems of the parasite, in the presence of a suitable stimulus, lead to a response which brings the parasite to a place where contact with a host can occur. In this category are the tactic and tropic responses of the parasite to thermal, gravitational, chemical, mechanical and photic stimuli and those behavioral responses of the parasite which lead to the establishment of a nutritional relationship with the host resulting in parasitization of that host. (Example: The swelling of the tentacles of snails from invasion by sporocysts of *Leucochloridium*. The altered tentacles attract birds, are ingested by the birds, and produce infection in them.)

B. *Adaptations for entrance to the host.* Once the parasite has come into contact with a host it must maintain its contact with the host externally (ectoparasites), or penetrate whatever barriers (*e.g.*, epidermal covering) separate them and thus enter the host organism (endoparasites). The mechanisms may be anatomical, physiological, or behavioral. External parasites may be provided with holdfast structures, tropisms for orienting them to the appropriate part of the host, or the more complicated behavioral patterns for adjusting to conditions antagonizing their persistence on the host. Internal parasites must be provided with both anatomical and physiological mechanisms for penetrating the integument and other tissues which separate them from the favored site in the host.

C. *Adaptations for persistence in or on the host.* Once established in or on the host, maintenance is effected in part by the further action of properties leading to establishment and in part by those properties of the parasite which protect from, give tolerance to, or result in avoidance of host mechanisms inhibitory or destructive to the parasite. Included in this category are structural features, such as those which make the parasite resistant to digestion or to antibody action, secretions which inactivate host inhibitory substances, and behavioral responses or alternative pathways which avoid host counteractions.

D. *Trophic adaptations.* Parasites frequently possess special adaptations for obtaining food from the habitat. These may include mechanisms for rasping, piercing, chewing or sucking, or modifications of body structure resulting in an increase in absorptive surface. The parasite must obtain essential nutrient materials. These may be special macronutrients or substances required for energy production or syntheses; carbohydrates and amino acids would be included in this latter category. Many animal parasites seem to differ from free-living animals in having absolute requirements for certain of these macronutrients. Carbohydrate is frequently required but some parasites lack the digestive capacity for degrading polysaccharides to a utilizable form. In their metabolism, animal parasites usually carry out incomplete oxidation of substances serving as energy sources and thus do not extract the total available energy from food molecules. Enzyme systems for complete oxidation of foodstuffs are frequently lacking in the protoplasm of parasites. The incompletely oxidized products are commonly excreted into the environment, usually in the form of free fatty acids. The incomplete oxidations common to parasites seem to be part of the general pattern that removal of selection pressure in a certain direction is followed by loss or partial loss of structure and function. Little is known of the metabolism of nitrogenous compounds by parasitic animals. Nitrogenous wastes are frequently excreted in the form of amines, amino acids and ammonia.

E. *Reproductive adaptations.* It is trite to say that any organism must reproduce in order for the species to survive. However, the reproductive process may be modified and specialized for the parasitic state. In counteraction to the increased hazards to survival resulting from the difficulties of reaching the one or more hosts involved in its life cycle and establishing and maintaining itself there, the reproductive capacity of the parasite is often very great. This may be accomplished in several different ways. The numbers of reproductive organs may be multiple, egg-production may be prodigious, or vegetative reproduction may occur in various forms and continue for prolonged periods, often with short generation times. With the possible exception of viruses, none of the types of reproduction in parasites can be claimed to differ basically from those in non-parasitic organisms. An increased reproductive capacity in parasites is common.

F. *Adaptations for transmission.* Highly diverse properties of parasites lead to transmission to another host although, in transmission by direct contact, there may be no obvious special properties at all. Furthermore, in those cases where a vector is concerned in transmission, vector activity may greatly influence the properties to be expected. Usually, however, there are 1) resistant stages such as eggs, spores, or cysts for contaminative modes of transmission, 2) properties for dispersal and penetration in some cases where additional hosts are involved and 3) properties for survival, or growth and multiplication, in the additional hosts where parasite entry is largely passive.

G. *Genetics of parasitic adaptations.* The part played by the heredity of the parasite has become well recognized for certain phytopathogens but, at present, is only inferential for animal parasites. In the former, characters influencing infectivity and pathogenicity are known, in some instances very precisely. In the latter, it can only be inferred from knowledge of some taxonomically related non-parasitic organisms that hereditary mechanisms influence infectivity and pathogenicity of animal parasites.

H. *Consequences of parasitism for evolution of the parasite.* Assumption of the parasitic way of life has certain effect on the subsequent evolution of the organism. The adoption of parasitism may be a process of isolation, with the parasite restricted to hosts which may be thought of as habitats. Adaptive modifications leading to a better fit of the parasite and the habitat have frequently rendered the parasite incapable of exploiting habitats other than those furnished by hosts. In extreme cases, adaptive modifications have resulted in the loss of capacity of parasites to live in or on more than one host species. Homeostasis of the habitat may result in selection of populations of genotypes having narrower ranges of adaptive capacities. Rates of evolution in the parasite will be conditioned by alteration of host anatomy and physiology and will thus be functions of the rates of evolution of the hosts.

III. The Phenomena of Parasitism

The essence of parasitism is the interaction of two living organisms. This interaction has given rise to consequences to each and to specializations of each. The specializations of the parasite have usually become fixed, i.e., are now inherited. We have to consider how these specializations may have come into being, how they are perpetuated, and what effects, beneficial or detrimental, they have on the individuals and on the species to which the individuals belong.

A. *Types of parasitic associations.* Parasitism may be categorized in various ways. The term symbiosis, as originally used, covers all types of associations between the host and the invader, including antagonistic and mutualistic interactions. This obviously covers many degrees of parasitism and has invited controversy over attempts to provide sharper lines of delineation. Mutualism may grade into commensalism which, in turn, may grade into parasitism. Parasitic associations may be categorized in terms of the proportion of the life history involving parasitism. It may include the entire life cycle or only some fraction thereof. Ecto- and endoparasitism are frequently not sharply defined. Likewise, the distinction between predation and parasitism is extremely difficult. These apparent complexities of intergradation are to be expected in the evolutionary history of biotic associations. The wide taxonomic diversity of parasites indicates that they have had many phylogenetic origins.

Levels of parasitism can be defined on several bases; especially pertinent are the nutritional or physiological and the morphological, by degree of intimacy of host and parasite bodies. These physiological and morphological relations apply whether the association is beneficial to both members or to only one. They apply correspondingly to mutualism and parasitism. The level or type of parasitism is often described in physiological terms as obligate, facultative, etc., or in morphological terms as extracellular, intercellular, intracellular, etc.

Intracellular forms are usually but not always microorganisms. They may occur in the cytoplasm of one type of cell, or in some cases, cells specifically adapted to contain them (mycetocytes), or in various cell types. Intercellular localization is common in infection of an organ, including organisms living in the blood. All of an organ may be involved, or parts of an organ, or special organs (repositories, especially for mutuals), or special growths (gall, tumors, etc.). Many ectoparasites really belong here as invaders of the integumentary system; they may be fixed, mobile or transient.

The whole animal may be concerned by invasion of all of its parts, or by products affecting the whole body of the host. Compound parasitism and mutualism are fairly common, that is, chains of parasites or mutuals, or mixed chains involving both. Finally, a society has been called a "supra-organism," and accordingly a species may be said to be a social parasite if it lives upon a society, or a social mutual if it exhibits a mutualistic relation with a society.

B. *Specificity of the host-parasite relation.* Parasites, like other organisms, show varying degrees of fastidiousness in the habitats they can populate. In parasitism, habitat limitation or preference is expressed in the intra-organismic (tissue distribution) and inter-organismic (host range) distribution of parasites. Of particular interest are the degrees of specificity in habitat selection and the mechanisms determining this specificity.

On the intra-organismic level parasites vary from extreme localization to almost complete non-specificity. At the cellular level specificity is of particular interest, involving extremely intimate relations between parasite and host metabolism. In part, of course, this area is of greatest significance for the clues it may furnish to problems considered elsewhere, such as the specific requirements of parasites and the adaptations which serve to satisfy these requirements.

On the inter-organismic level the range is from one-host specificity to very wide host distributions which may exceed the boundaries of classes or even of phyla.

The mechanisms affecting intra-organismic specificity, or localization within the animal host, are poorly understood, although certain factors are partly known and others may be reasonably presumed to operate. Thus, specific chemotropic responses of the parasite may direct it to particular sites in the host. On the other hand, the dispersal of the parasite within the host may be weakly determined, being even largely random, the final localization depending on the capacity of particular cells, tissues, lumina, etc, to satisfy the nutritional requirements of the parasite. Conversely, inimical chemical properties of individual sites, such as secretions at particular levels of the intestine, may inhibit or destroy the parasite. In some cases available routes of migration within the host may be determinant. The methods and portals of access to the host from the outside thus combine with the pathways inside the host to permit arrival in some sites and to bar entrance to others. Clearly, combinations of the types of factors discussed here must occur, so that pathways to a site, responses of a parasite to stimuli along these pathways and the environmental suitability of the site may combine to create complex and highly specific tropisms.

The mechanisms affecting inter-organismic specificity, or limitation to particular groups of hosts, is determined by both opportunities for contact with hosts and the capacity to invade and survive in hosts if the contact is made. Both types of factors determine the natural distribution of parasites; only the latter affects experimental susceptibilities, since the opportunity for contact may be deliberately supplied in the laboratory. In nature, opportunities for contact with hosts are limited by any spatial, temporal or behavioral separation of the host and parasite. Thus, a parasite obviously cannot reach a host from which it is widely separated geographically, whether the geographic separation is a result of accident, of different requirements of the two in terms of the external environment, or of environmental separation of the host in question from other hosts of the parasite. On a smaller spatial scale, any ecological boundaries may operate in the same way. Similarly, seasonal or even diurnal unavailability of a particular host may make it inaccessible to a parasite whose activities are limited by such factors. Behavioral properties of the host, such as gregariousness, selection of defecation sites, coprophagy, food requirements, etc, may separate the host from the parasite, even though spatial or temporal barriers do not exist. Among the factors which may operate are quantitative ones; thus, it is not merely the qualitative availability of a host species which determines whether a parasite may utilize it, for the density of the host species in a particular area can have equally important results.

Capacities to invade and survive in a host may involve any of the requirements and susceptibilities of the parasite. Structural and mechanical properties of integuments of some hosts, for example, may bar the access of certain parasites. The physical characteristics of tissue sites, such as pH, redox potential or even such simple elements as the viscosity of tissue fluids, may fail to suit the requirements of the parasite. Different animals show great differences in kind, intensity and even chemical specificity of acquired immune responses. Specific chemical attractants elaborated by particular hosts may affect the orientation of parasites to them. Lethal or inhibitory chemical factors may specifically bar certain parasites: Examples may be found in the bactericidins of certain diptera and the lytic properties of the blood plasma of particular vertebrates. One of the most important factors involves the particular chemical requirements of parasites. Nutritional and other

needs of organisms being often complex, it is not surprising that they are likely to be satisfied by only certain types of hosts.

Specific chemicals present as metabolites in the host or produced by the stimulus from the parasites may affect the compatability of the relationship. Finally, the existence of other parasites in or on a host may greatly affect the ability of a parasite to survive. There is suggestive information on this point with respect to some ectoparasites and abundant reason from general ecology for the supposition that competition between parasites in or on a host may be as important as it is for free-living organisms in any habitat.

C. *Consequences for evolution of the host species.* Parasitism may be considered as an evolutionary pressure. The living together of two organisms has the effect of making these a unit in nature. This is particularly true when the association is obligatory for both partners in the association. In such cases there is an effect on the potentialities of the host to occupy a particular environment or even to survive. In numerous cases the effect is detectable only under conditions such that the host without symbiotes is subjected to stress—but such conditions occur repeatedly in nature.

When the association is that of a host and parasite where only the parasite benefits, the effect on the host can be only detrimental. The effect can be negligible on the host, or evident only under adverse conditions such as malnutrition, or regularly evident as lowered vitality, lowered reproduction, slower growth, or loss from the population of the infected individuals. Such lowering of vitality has an effect on survival comparable to that of a semi-lethal gene, i.e., it has negative survival value. In the course of evolution it could have, and presumably has had, the effect of eliminating a species since reduction of population size below a certain minimum generally leads to extinction. This can lead to change in a population by selection of resistant individuals or more resistant species. Obviously, extinction of any host is automatically accompanied by extinction of its specific parasites. Since groups of parasites often evolve within groups of hosts, the relations between the parasites of two or more hosts may indicate relations between these hosts. This type of evidence has at times been used in taxonomic and zoogeographic studies on host groups the phylogeny of which was not clear.

In the association of a host and a mutual where both partners benefit it would seem that the association should be beneficial to the population. Correlations and laboratory studies have shown that possession of mutuals permits some hosts to live on foods which they could not live on without their symbiotes. In other cases an effect is demonstrable only under stress conditions; it then has a positive survival value in terms of evolution. In some cases no deleterious effect has been demonstrated from elimination of the supposed symbiotes. Alternately, a host might develop the ability to do some of the same things. In some cases of mutualism there has apparently been selection of such specializations that neither host nor symbiote could now live without the other.

There is some evidence of host adaptations to parasitism. Hosts may change, presumably by selection pressure, to give strains better adapted to resist or tolerate parasitism. The evolution of antibody responses to antigens and the organizational specializations of the lymphatic system in the vertebrate represent such adaptations. In mutuals, obvious adaptations are the development of special abodes, such as

mycetomes, and arrangements for transovarial infection or other means of assuring that all progeny receive the symbiote.

Our knowledge of the role of genetic control in the host-parasite interaction is limited largely to the highly specialized or restricted types of parasitism. At present the best examples are found in the plant pathogen-host interactions and have been defined as factors conditioning pathogenicity (virulence and avirulence) in the parasite and those conditioning the resistance or susceptibility reaction of the host. Presumably some genetic control of host-parasite interaction functions in all parasitism, although more detailed investigations are necessary to establish the relation. Excellent examples of the genetic interaction between parasite and host are found in the cereal rust diseases, apple scab, and potato late blight. Both gene mutations and regular Mendelian gene segregation and recombination have been demonstrated.

D. *Consequences of parasitism for the host population.* Parasitism has certain consequences for the host population. These may be manifested most dramatically by fluctuations in numbers caused by epidemics. Epidemics can give population fluctuation curves quite similar to ones given by predator-prey fluctuations. Biological control of pest insects provides many examples in addition to the epidemics that regularly ocur in nature. In this case control of the host is considered desirable rather than unfortunate. Parasitism can affect the age composition of a population. This is particularly true for common diseases to which all surviving individuals develop complete immunity.

E. *Effects of parasitism on the host individual.* Obviously, parasitism would have certain consequences for the host individual. There are many cases in which the parasite has no *detectable* effect on its host. The relation may thus be beneficial to the parasite and apparently neutral with respect to the host. When effects are discernible, they may be injurious or defensive, or, in the case of mutuals, beneficial.

(1) *Injurious effects.* Injurious effects show a wide range of severity. The severest produce frank disease. Others, such as hookworm, may affect the entire population thus making disease in individuals less apparent. Less severe effects may result in relatively unimportant injury to the host or in damage which is kept in essentially full repair by the host. Mechanisms of injury are extremely varied, and it is noteworthy that some types are illustrated better by animal parasites than by other types of pathogens.

Mechanical injury is common, involving such destructive actions as perforation, rasping, piercing or chewing or obstructive effects, such as obstruction of a lumen or blockage of chemical transfer across cell or tissue membranes. The mechanism of disruption of cells by the growth of intracellular parasites is obscure, but, in some instances, this may be a result of mechanical injury.

Chemical injury may involve specific toxins (not clearly demonstrated in animal parasites) or less specific components or excretions of the parasite which adversely affect the physiology of host cells. The nature of the injury to the host cell may be lethal, inhibitory or stimulatory. The last is of special interest. It may involve hypertrophy, hyperplasia, or, in a few cases among animal parasites and many among viruses, neoplasia. The benign neoplasms induced by some parasites not uncommonly develop into malignant ones. In some examples, as in the induction of galls by insects, the hyper- or metaplasia may be beneficial to the parasite.

Parasites may rob the host of essential body components, as in the case of blood-

sucking forms. Related to this type of action is that of competition with the host for nutrients. The accumulation of vitamin B_{12} by *Dibothriocephelas latus* would seem to belong in the latter category.

Particular interest attaches to those chemical actions which result in harmful immunological responses by the host, the hypersensitive reactions. Elaboration of this area probably belongs with consideration of immunological responses of the host as defensive reactions, but it should be emphasized here that there are injurious aspects to many of the host's "defensive" reactions, and that some of them seem to have no protective value whatever.

As with many of the other phenomena of parasitism, viruses may occupy a unique position with respect to pathogenesis. They often participate so intimately in the metabolism of the host cell that physiological processes are modified in very subtle ways, by altering synthesis, binding or utilizing substrates, etc. In most respects these activities may be thought of as consequences of the reduction of action to subcellular levels, but this quantitative reduction may have what are essentially qualitative effects.

Finally, parasites may injure a host indirectly, by conveying to it other, more harmful parasites. Most of the examples are to be found among ectoparasitic hosts of pathogens, but there are cases in which endoparasitic worms act as vectors of viruses and rickettsiae.

It is worth noting that the location of injury may often be critical for the host. An extreme case is furnished by parasitic castration of individuals or societies, where the reproductive organs or individuals are specifically destroyed. It should be recognized that the harmful effects of parasites may involve combinations, serially or simultaneously, of two or more mechanisms. Furthermore, one injury may set off another injurious reaction. Thus, destruction of erythrocytes by *Plasmodium* may release components of these cells which are harmful to others.

(2) *Defensive reactions of the host:* The *ad hoc* defensive reactions of hosts to invasion by parasites are important aspects of host physiology, serving to emphasize the great significance of parasitism as a problem with which all organisms must cope. Reference is made here not to pre-existing barriers, chemical incompatibilities, etc., however dynamic, but to those defenses which are mobilized in direct response to the presence of the parasite. In some situations these reactions may be more effective in "abnormal" than in "normal" hosts so that they affect host specificity.

Inflammatory responses are often the first reaction to invasion by parasites. Inflammation may be general, involving multiple sites of cellular reaction. More often it is localized in subcutaneous tissues, lung, lymph nodes, spleen, etc. The response is primarily cellular, although humoral contributions are usually also involved. The inflammatory process is commonly divided into the relatively distinct, but normally overlapping, stages of (1) temporary localization and destruction of parasites, (2) walling off or encapsulation of the invaded area, and (3) repair. While the process often has drastic effects, as in pneumonia, it is basically beneficial, protecting the rest of the host body from parasitic invasion.

Antibody responses constitute major secondary defenses against parasites. Antibodies may destroy, localize, or neutralize parasites or interfere with their reproduction. They may participate in the inflammatory response by enhancing

the phagocytosis of invaders. They may bind or neutralize toxins elaborated by parasites. The remarkable specificity of antibody production and of antigen-antibody reactions has stimulated more wide-spread interest in them than would be justified by their relationship to parasitism alone. Indeed, there is hope that an understanding of the physiology of antibody-formation will lead to clarification of some of the exceedingly specific synthetic mechanisms which are such a fundamental property of life. Noteworthy also are the adaptations for transfer of antibodies from mother to young in the higher vertebrates, for they testify to the ubiquity of parasitism as a condition of life. Hypersensitivity deserves mention as an unfortunate corollary of the antibody response.

Although earlier papers reported the production of antibodies by plants, the evidence is not generally accepted. Non-specific, anti-bacterial substances are said to be produced in some invertebrates as a result of infection.

Some of the apparently harmful effects of parasitism may have long-range value for the host. Necrosis or infarction of cells and tissues may operate to limit the spread of parasites beyond the injured area. Perhaps related to these phenomena is the deposition in the cell walls of plants of compounds which inhibit the passage of pathogens.

Various more or less conscious defensive reactions are observable. A host may exhibit definite evasive action, as in the flight of cattle from bot flies, or it may actively pursue and destroy parasites by preening or other methods. An interesting adaptation of some hyperparasites is connected with these phenomena, so that cestodes, for example, may be conveyed to the definitive host by ingestion of an ectoparasitic intermediate host. Deliberate control measures carried out by man are, of course, a special case in this category.

Finally, the host may react to parasitic injury with mechanisms of repair, regeneration or compensation for damage. The enhanced erythropoiesis seen in infections producing anemia is an example. Also included are the direct repair of injuries caused by parasites, as to the intestinal mucosa, and the reconstruction of tissues damaged by inflammatory responses.

In higher animals many of the responses discussed above are functions of the mesenchyme. These potentialities of a tissue, which maintain in the adult animal a high capacity for alternative differentiation, illuminate important features of host biology.

(3) *Beneficial effects—Mutualism.* The beneficial effects observed in mutualism may be facultative or obligatory. An outstanding classical example is the facultative mutualism shown by lichens. In some cases, both fungi and algae may grow readily alone with suitable conditions but are incomparably more successful when together.

Presumably in all cases, endomutualism or endosymbiosis is potentially facultative in the sense that, given the right environment and nutrients, the partners could exist alone. However, in some cases the specialization is so great that the partners could not exist in any natural environment, and in many cases the benefits are such that the normal natural diet of the host is inadequate without the symbiote. Thus, especially among insects, symbiotes may digest foodstuffs such as wax, cellulose and chitin which would otherwise be difficult to digest, or supply vitamins and other nutrients such as amino acids. As far as the host is concerned all of the bene-

fits are expressed in mechanisms of metabolism (digestion, nutritional requirements and possibly mobilization of compounds such as urates). As for the microorganism, the host obviously supplies a satisfactory environment plus metabolic precursors; the host also provides an abode, geographic distribution, and usually a mode of transmission. However, the host may become so unsatisfactory to the symbiotes at certain stages as to cause their death, thus changing the relationship to one of predation. There are only a relatively few documented cases of mutualism but many cases of logically presumed mutualism (including presumed microorganisms). It is not safe to assume that the benefits the host obtains are the sum of what the microorganism can do *in vitro*. Thus, certain symbiotes fix atmospheric nitrogen in culture flasks but do not do so in the presence of organic nitrogen and have not been shown to do so in nature. Also, evidence indicates that cultured microorganisms may rapidly undergo adaptive mutation and hence give misleading data.

When the mutualism does not involve one organism inside of another the benefits are various. One species may be a feeding place for another species which feeds on ectoparasites. One species may be furnished a place to lay its eggs while the other species has sexual reproduction dependent on the activities of the first. One species may obtain protection and transport while the other obtains food as a product of the association.

IV. Relation of Parasitism to Human Welfare

Although the principles outlined above concern the biology of parasitism without emphasis upon those aspects which directly affect man, nevertheless understanding of these phenomena has utilitarian value.

The most commonly recognized examples of parasites are pathogens, those which produce disease in man and in animals and plants, both domestic and feral. Thus the understanding of parasitism is important in the fields of medicine and public health, agriculture, conservation, and aquatic resources. The control of pathogens in populations requires understanding of the principles governing the life cycle and spread of these organisms. The understanding and utilization of these principles in interrupting the activities of the parasites constitute the goal of epidemiology. If one can find and break the weak link in the chain of events that produces an epidemic then he can control the epidemic. An element in successful control of the pathogen may be the curing of infection in the individual.

In a few cases mutualistic relations between organisms, chiefly in agriculture, are of some benefit to man. These relationships may, however, have intergrading types with those producing harmful effects. In certain instances the introduction of parasites serves as a factor in reduction of populations of pests or pathogens.

DEVELOPING THE COURSE

In designing a course in parasitism or animal parasitology one must consider the level at which it should be given, what the course should try to accomplish, practical matters of time and facilities, and the competitive merits of the varied approaches possible to the subject. This section of the report presents our thoughts on these questions and leads to the presentation of a few sample course plans.

We start with the presumption that students taking the course will have had at

least a year of biology and a year of chemistry, but more of both would be highly desirable. Prerequisites should not be set so high as to bar interested students from this basic course in inter-organismic relationships but must assume the fundamental knowledge contained in good first courses in biology and chemistry.

I. Objectives

The course should be visualized as providing a broad biological appreciation of associations between organisms for all students interested in the biological sciences and as a common basis for students intending to go on to special curricula in fields such as medicine, microbiology, public health, veterinary medicine, and wildlife management. It is recognized that parasitic and other symbiotic relations are a part of ecology and capable of being incorporated into a larger course in ecology if desired.

The course should stimulate students to think in terms of fundamental principles and encourage further elucidation of biological principles. This can be accomplished in no cut-and-dried fashion but rather through stimulating the student to independence of judgement, to self-motivation, and to self-reliance. Like any good course, this one should make the student keenly aware of the vastness of the unknown. The course should cover the principles set forth in the foregoing précis.

The intent of the types of courses considered requires a deliberate concentration on the teaching of principles as *the* major objective. Other objectives should be firmly subordinated to this one but should, of course, be taken into account wherever they do not interfere with it. Design of a course, furthermore, demands concern for the treatment of whatever supra-principles exist in the subject matter. Thus, the individual principles considered in the preceding précis might very well be presented in relation to individual aspects of the biology of a great variety of organisms, but unless a student sees rather comprehensively a parasitic association in action he may fail completely to grasp the main ideas of parasitism.

There are certain intellectual obstacles to the design of courses of the type we are advocating. For example, teachers very legitimately fear the detachment from facts which endangers any course based on principles; a course must keep down to earth lest the student lose contact with reality.

Another difficulty frequently blocks good course design, particularly in areas with important practical aspects such as parasitology. A student who has had a course in parasitology is commonly expected to have some technical competence in the field, hence the course plan often involves serious compromises intended to give the student information which someone else thinks he should have. It should be clear that the type of course under discussion in this report is an introduction to the concepts of parasitism. It is not meant to meet technical needs, since practical competency in parasitology or any other field of parasitism would demand considerable further study.

The selection of illustrative material involves many extraneous considerations. The use of examples which have special practical significance or unusual interest, for example, can have pedagogic value and at the same time meet secondary objectives. There should be clear recognition of the hazard that practical aspects can obscure the fundamentals. Similarly, other things being equal, it can be desirable to select as examples groups of organisms with which a student should acquire some

familiarity. Considerations like these may demand compromises in course design. The important point is that such compromises be made knowingly and without significant detriment to the primary objective of the course.

Within the framework of the preceding concepts a course with the emphasis on principles of parasitism may be successfully taught in a variety of ways. The members of this panel have confidence in the ability of teachers to design courses to meet the primary objectives here suggested and fitted to the level of training of the students to be taught as well as to the available time, physical equipment, and biological materials. We also realize that in some instances circumstances may make necessary different degrees of compromise with the ideal to which our précis points. With these thoughts in mind we offer below brief descriptions of a few sample plans of some of the ways the course might be given. It is hoped that these few samples will suggest others, and that from the various courses which are possible under the local conditions the instructor will be able to select from all of these the one which most closely meets the primary aim emphasized by the preceding discussion.

SAMPLE COURSE PLANS

Sample A.

Intensive study of selected material followed by a more comprehensive survey. In this plan a small number of examples, perhaps 6–8, would be chosen for their appropriateness in exhibiting as many of the principles of parasitism as possible. These would be given study intensive enough to furnish the student with a very good understanding of the particular principles which they were especially suited to demonstrate and to furnish him with a large part of the terminology and the conceptual familiarity necessary to the more extensive study of examples which would follow.

Since many factors will enter into the choice of examples to be used in the intensive study the best that can be done here is to offer some criteria for their choice. It would appear advisable to choose examples to include as many as possible of the following:

(a) monoxenous life-cycle
(b) heteroxenous life-cycle
(c) intracellular parasite
(d) extracellular parasite
(e) endoparasite
(f) ectoparasite
(g) life-cycle involving a vector
(h) life-cycle without a vector
(i) harmful parasite
(j) mutualistic relationship
(k) examples from the major groups of hosts and parasites.

One combination of hosts and parasite may involve many of the above categories; for example: malaria, *Leishmania donovani*, and *Trypanosoma cruzi* will illustrate categories *b, c, d, e, f, g,* and *i* above and, therefore, a choice could be made from among them. Other combinations of the categories result from other possible combinations of host and parasite.

Circumstances may indicate the desirability of including exercises involving plant parasitism. The following are listed as suggestions:

(a) Inheritance of pathogenicity by (1) the apple scab fungus, (2) the corn rust fungus or (3) the potato late blight fungus.

(b) Inheritance of resistance by onions against onion smudge fungus because of protocatechuic acid.

(c) Pathogenicity of *Pseudomonas tobaci* because of the amino acid tabtoxinine (*J. Biol. Chem.* **198:** 807–813, 1952).

(d) Pure culture of potato nematodes on tissue cultures of potato tissue.

(e) Symbiosis of legumes and the nitrogen-fixing legume-root-nodule bacteria.

Thus with careful selection of parasites and hosts a very representative coverage of the principles of parasitism and at the same time a fairly wide selection of taxonomically different hosts and parasites can be obtained. It should be emphasized that selection of the material is to be primarily based on their value in demonstrating principles.

After the above study has been completed a portion of the remaining time may be devoted to examining less intensively a larger number of examples which represent principles not found in the first ones. Analysis of the principles represented by these additional examples can be done more expediently following the experience gained from the intensive study.

A final portion of the course should be devoted to integration and synthesis. Not only should the over-all concepts represented in the précis be developed and related but the unsolved problems in the various fields involving parasitism should be recognized. At this time orientation can be given to the more specialized courses to be taken by the student subsequently.

Sample B.

Principles of parasitism emphasized in the lectures; laboratory nearly independent. The principles outlined in the précis are covered by lectures and discussions with cross reference to examples studied in the laboratory whenever relevant and when already covered in the laboratory. The lectures (or discussions) deal with the principles which may be either stated positively as in the "précis" or rephrased into questions such as "Why study parasitism?" "What is a parasite?" "What are the properties of the environments of different parasites?", etc.

The laboratory series covers selected types of parasites, some life cycles, and some properties illustrating certain of the principles. Scheduled as a 15-period sequence, the following scheme may be used with some modification from year to year depending on what living material happens to be available.

1st period: Survey of animal kingdom and of parasite groups; demonstration and refresher.

2nd period: Malaria—mosquito anatomy and life history.

3rd period: Malaria—*Plasmodium*, full life-history preferably with some living material.

4th period: Relation of *Plasmodium* to hosts. (Substitutes for 2, 3, and 4: *Leishmania donovani*, *Trypanosoma cruzi*, or Filaria)

5th period: A nematode (*Nippostrongylus*, or hookworm) or a schistosome—anatomy, life-history and relevant taxonomy.

6th period: Same worm as used in period 5.—pathology of host; worms in culture.

7th period: Amebiasis or trichomoniasis—emphasis on extracellular nature; interaction of host and parasite; means of transmission.

8th period: Mutualistic protozoa of termites (or of the wood roach, *Cryptocercus*). Demonstration of living flagellates in gut, study of inclusions in the flagellates. Additional or alternative material: Symbiotic bacteroids in cockroaches; lichens.

9th–11th periods: Experiments with living parasites depending upon their availability (properties of the digestive tract, tolerances to digestive fluids, excystation, comparative histochemistry, production of antibodies, culturing, temperature tolerances, oxygen requirements, etc. depending on what the instructor has as equipment for student use and with which he is familiar).

12th period: Other parasitic protozoa and the smaller phyla (*e.g.* the Acanthocephala). Emphasis on diversity.

13th period: Parasitic nematodes. Emphasis on examples grading from free-living to parasitic.

14th period: Parasitic flatworms. Emphasis on grading from simple to complicated life cycles.

15th period: Parasitic arthropods. Emphasis on the relation to environmental factors. If desired, some control ideas may be included, or with given data, the students may be required to attempt an epidemiological analysis.

Sample C.

"Principles" emphasized in both lecture and laboratory. In the laboratory work an attempt is made to sample the principles. For this work there is also the presumption that in lecture or discussion and required reading, specific reference will be made (1) to the phyla containing parasitic species and (2) to some sample life cycles. A largely subjective type of examination would be given but some kind of practical examination on the work of the laboratory would also be given. Instructors will recognize that care is needed to avoid excessive fragmentation of material in a course of this type.

Laboratory Period 1. Orientation. Emphasis upon (1) similarities and differences between the free-living and parasitic forms, and (2) the host as a habitat. The laboratory work could be combined with a field collecting trip to obtain materials from a habitat or could use animals from an aquarium in the laboratory. Compare a free habitat with the alimentary canal of a vertebrate and its content of parasites. Stress fitness for the habitat in both cases.

Laboratory Period 2. Orientation. A series of about 15 associations assembled on demonstration and fitted to suitable explanatory material. To include plant-plant, plant-animal, animal-plant and animal-animal associations. Also mutual, commensal and parasitic. From these the

student arrives at answers to a series of questions leading him to tentative decisions on definitions.

Laboratory Period 3. Orientation. Parasitism of a single host. Choose a host known to have a variety of parasites in a single individual (or partially experimentally induced). Class makes survey for parasites. Host as an "apartment house" for many "guests" in a variety of habitats in a host. Such examples of technique as desired could be added (fecal smears, organ presses, blood smears, dissection, permanent slide making). In frogs one usually finds lung flukes, bladder flukes, nematodes, filaria, and intestinal protozoa. Rat, cockroach or earthworm or examples with which the instructor is most familiar can be substituted for or used as supplements to the work on the frog.

Laboratory Period 4. Behavioral properties for reaching the host. A central theme for intensive work plus additional demonstrations for comparison and from which answers to posed questions may be obtained. Suggested example: a snail species and two or more kinds of cercariae emerging from it, those with and those without tails, with and without eyespots, etc. Observations on structure are compared with reaction of the cercariae to light, types of movement, etc. Relate behavior to capacity for making contact with the host. (Alternative material for laboratory or demonstration: Nematode larvae from culture (gravity), ticks on a pencil or blade of grass (temperature), decaying flesh and blue bottle flies (olfaction), mite from mantle cavity of fresh-water mussels (light and chemoreception), leeches (mechanical stimuli)).

Laboratory Period 5. Survey of holdfasts and other mechanisms for maintaining position in or on the host. Emphasis and detailed study on 1 or 2 forms with others on demonstration for comparison. Scolices of tapeworms, suckers and hooks of leeches, trematodes, crustacea. Crab louse *vs.* mosquito. Anterior ends of nematodes. Cephaline gregarines.

Laboratory Period 6. Parasite resistance against or toleration for host. Example: any intestinal nematode in artificial digestive fluid. Compare the intact worm with worm cut open or in pieces. Blendorize trichinous muscle and put in pepsin + HCl. Or test cercaricidal action of a variety of normal sera (cat serum as control). Or agglutination of *Trichomonas foetus* by various normal sera.

Laboratory Period 7. Trophic properties. Use period to demonstrate one or more of the hydrolytic, respiratory or synthetic capacities of parasites. This is where the instructor's ingenuity is needed. It is not so important what material is chosen but how it is used to stress the point to be made. It can be a laboratory period on morphology or physiology, but, if morphology, not for its own sake. Could use the intestinal amoebae of man stressing the different structures as results of different synthesis, or histochemical demonstration of RNA (volutin granules of trypanosomes) or DNA, glycogen in protozoa or tapeworms, glucose utilization by trypanosomes.

Laboratory Period 8. Reproductive apparatus and products. Possible examples: *Clonorchis*, coccidia, malarial parasites (sexual and asexual cycles), bladder fluke of frog, or *Schistosoma* in mouse.

Laboratory Period 9. Transmission to another host. Helminth eggs: *Enterobius* or hookworm for direct and indirect contact, *Ascaris* or *Echinostoma* for food and water contamination; supplemented by filarial transmissions by Diptera.

Laboratory Period 10. Consequences for the host. Have the student find and describe from slides destruction of red cells by malaria parasites, of intestinal cells by coccidia, distortion of host cells by *Leucocytozoon*, or see gross and microscopic liver pathology from schistosome infections of mouse, amebic infections of rat or human, effects of hookworm attachment on mucosa, walling off of foreign bodies (as in *Echinococcus*). Immunologic effects could be illustrated here instead: agglutination or lysis of protozoa, precipitate formation around nematode larvae. The beneficial effects of algae on *Paramecium bursaria* could be studied here as an experiment in physiology of beneficence (capacity to survive in a bacteria-free medium in presence of light *vs.* capacity to exist in the dark in the presence of suitable bacteria). Or the protozoa of termite or wood roach would be effective (built around their identification) if a series of questions led to establishing the stress desired.

Laboratory Period 11. Evolution. Of either host or parasite. Host evolution to phagocytosis as a defense mechanism or lymphoid tissue and antibody formation. The evolution of blood-sucking in the series *Tabanus* through forms like *Musca* to *Stomoxys* and *Glossina*. Parallel evolution of hosts and parasites as evidenced by speciation in trematodes and acanthocephala.

Laboratory Period 12. Interorganismic specificity. Examples: Contrast *Entamoeba* in intestine with coccidia (*Eimeria* largely in birds, *Isospora* in mammals); lack of specificity in *Trichinella*.

Laboratory Period 13. "Levels" or "degrees" of parasitism. Intracellular to intestinal lumen inhabiting: Trypanosomidae. Dermal to intestinal: *Gasterophilus*. Simple to complex: nematode life-cycles.

Laboratory Period 14. Further interactions. Arthropods as hosts and influence on epidemiology. A laboratory exercise developed around mosquitoes, ticks, mites, fleas, lice to establish the significance of development and multiplication of the parasite in the vector in relation to epidemiology. Alternate: virus transmission in plants by aphids.

Laboratory Period 15. Parasites and human welfare. A diagnostic exercise stressing the role of identification and taxonomy in chemotherapy and control: stool analysis for amoebae, identification of fly maggots or mosquitoes, helminth eggs, blood smears for trypanosomes or malarial parasites.

OTHER SUGGESTIONS

We believe that the teaching of parasitism would profit from more frequent and more systematic exchange of ideas and course plans. It is hoped that this report will encourage our colleagues to write other teaching studies. We also think that lists of materials and reading useful in teaching parasitism would be very helpful. Individuals or groups who prepare such lists can do their subject an important service. In particular, we suggest the need for—

1. Lists of plant and animal materials
   a. Comprehensive list of host-parasite systems
   b. A critically selected list of relatively well-known forms that exemplify basic principles and are readily available. The list could give information on how material can be obtained, culture methods, and uses in teaching.
2. Sourcebook of experiments and laboratory studies.
3. Lists of publications useful in teaching:
   a. research papers
   b. reviews
   c. monographs
   d. books
   e. historical references

Finally, *we want to re-emphasize our conviction that only the individual teacher, instructing a particular group of students on his own campus, can decide what his course should try to do, what it will include, how it will be organized. We have ventured only to offer some ideas and suggestions in the hope that they will help those who teach courses in parasitism or write textbooks and will encourage continuous critical review of his teaching efforts by every instructor in our field.*

RESEARCH NOTE

THE FISH PARASITE *ARGULUS LATICAUDA* AS A FORTUITOUS HUMAN EPIZOON

In June 1955 I was called upon to remove a foreign object from the eye of a 10-year-old boy who was suffering intense discomfort. The irritant, a parasitic copepod, was moving vigorously about on the surface of the eyeball. The copepod persistently resisted dislodgment, but was finally detached and preserved for identification. According to his account, the patient had been struck while swimming open-eyed in the clear inshore waters of the Tred Avon River (average summer salinity, 12 o/oo) off Oxford, Maryland. Several days later while crabbing across the river from Oxford, I captured a toadfish, *Opsanus tau*, which bore several argulids externally. These copepods were also preserved.

The argulids from human and fish were tentatively identified as *Argulus laticauda*, an ubiquitous ectoparasite of North Atlantic fishes. This identification was confirmed by Dr. David G. Causey of the University of Arkansas. Numbers of toadfishes are present in these inshore estuarine areas and it seems likely that the copepod had been attracted to the first moving object after accidental dislodgment from its normal fish host.

Although a single occurrence of this nature is of little general parasitological or medical consequence, it is believed noteworthy as a rare record of a parasitic copepod occurring on a human, albeit temporarily. (Contribution No. 77 from the Virginia Fisheries Laboratory.)—WILLIAM J. HARGIS, JR., *Virginia Fisheries Laboratory, Gloucester Point, Virginia.*

STUDIES ON MALARIA IN CHIMPANZEES. V. THE SPOROGONOUS CYCLE AND MOSQUITO TRANSMISSION OF *PLASMODIUM VIVAX SCHWETZI**

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Plasmodium vivax schwetzi, the natural benign tertian malaria parasite of chimpanzees, is known by its erythrocytic schizogony and gametogony and by its sporogony in *Anopheles maculipennis atroparvus* (Rodhain and Lassman 1940 and Rodhain 1955). There is no record of transmission of the parasite by the mosquito, nor is its exo-erythrocytic schizogony known.

The present investigation was undertaken to elucidate these 2 points and to determine if any characteristics existed in the life cycle of the parasite which would constitute specific differences to the similar stages in the life cycle of *P. vivax vivax* of man.

It has not been possible to demonstrate the tissue phase of the parasites and this communication is confined to the mosquito phase.

MATERIALS AND METHODS

The strain of *P. schwetzi* employed was found in Chimpanzee No. 22 after splenectomy. The vertebrate hosts employed were *Pan troglodytes verus* and the invertebrate hosts *A. gambiae* drawn from an old-established laboratory colony. The chimpanzee (No. 19) used experimentally was treated with 900 mg. chloroquine *base* for 3 days and 105 mg. primaquine for 14 days 2 months prior to experimental use.

All blood films were stained by the Giemsa methods. The chimpanzee was infected by the intravenous inoculation of infected salivary glands of *A. gambiae*. These were suspended in a medium of 20% (by vol.) inactivated human serum in Locke's fluid.

The study of oocysts was made on mosquito mid guts floating in physiological saline without the addition of fixatives. Care was taken to ensure that the cover-glasses used did not compress the gut or the oocysts. Mosquitoes were kept at room temperature (75°–80°F.)[1]

RESULTS

Chimpanzee No. 22 (estimated age 2 years) on reception showed a scanty infection in the blood with *P. malariae*. Following splenectomy an occult infection with *P. schwetzi* flared up and quickly exceeded the *P. malariae* infection. 100 *A. gambiae* were fed 7 days and another 100 *A. gambiae* were fed 10 days after patency of the *P. schwetzi* infection when numerous microgametocytes were seen. The 4- and 5-day-old oocyts were studied in these 2 batches of mosquitoes.

Fourteen days after the first batch and 11 days after the second batch had fed, the salivary glands of 104 surviving *A. gambiae* were inoculated into Chimpanzee No. 19 (estimated age 1½ years). No sporozoites could be found in the inoculum.

Chimpanzee No. 19 showed parasites in the blood 18 days after inoculation and the infection rapidly built up to a high parasitemia, due to *P. schwetzi*. Parasite counts rose to a maximum of over 100,000/cu.mm of blood. The only notable

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[1] I am indebted to Dr. R. C. Muirhead-Thomson for assistance in the dissection of mosquitoes.

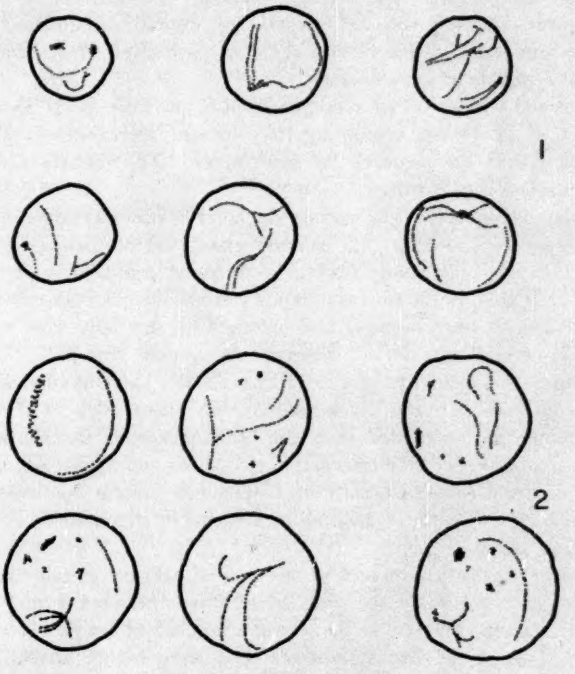
feature of the parasitemia was the high proportion of oval host erythrocytes. The merozoite numbers ranged from 18 to 24 per schizont.

150 *A. gambiae* were fed on Chimpanzee No. 19 when 260 microgametocytes/cu.mm of blood were present and an additional 50 were fed when 1070 microgametocytes/cu.mm of blood were present. The progress of the parasite in these mosquitoes was studied at all stages.

Sporogony of *P. schwetzi*

Normal microgamete-formation took place in the gut of *A. gambiae*. A very high concentration of ookinetes was found in the gut 21 hours after feeding. The ookinetes are largely vermicular, although some retort flask shapes can be seen.

The vermicular ookinete is between 15–28 microns long and one end is distinctly more pointed than the other. This pointed anterior end usually contains the golden brown pigment as a diffuse mass. The nucleus is large and placed 2/3rds of the length posteriorly. There is usually a central perfectly spherical vacuole containing a material which stains pink with Giemsa. The cytoplasm stains blue (posteriorly) to mauve (anteriorly).



EXPLANATION OF FIGURES

FIGURE 1. The drawings show the pattern of the pigment in oocysts of *P. schwetzi* 4 days after the infective feed. ×1000.

FIGURE 2. The drawings show the pattern of the pigment in oocysts of *P. schwetzi* 5 days after the infective feed. ×1000.

The oocysts were studied when 3 days old to 11 days old. The sizes given below are in microns.

The 3-day-old oocyst:—The average diameter (12 oocysts) is 11.1 (9–13). The pigment is distributed all over the cyst.

The 4-day-old oocyst:—The average diameter (28 oocysts) is 15.4 (11–18). The pigment distribution is illustrated in Figure 1.

The 5-day-old oocyst:—The average diameter (152 oocysts) is 21.8 (15–26). The pigment is visible in most and the pattern is illustrated in Figure 2.

The 6-day-old oocyst:—The average diameter (10 oocysts) is 31.2 (27–37). The pigment is no longer visible.

The 7-day-old oocyst:—The average diameter (38 oocysts) is 40.0 (28–56). Some slower growing oocysts can be discerned.

The 8-day-old oocyst:—The average diameter (74 oocysts) is 54.1 (49–60). In these figures definitely retarded oocysts are ignored. Loculation of the cytoplasm, septa-formation and the earliest signs of sporozoite-formation are all visible. The pigment is visible in some oocysts.

The 9-day-old oocyst:—The average diameter (87 oocysts) is 55.6. The average diameter of 50 oocysts containing fully formed sporozoites is 57.0 (47–65). The salivary glands are negative for sporozoites. The oocysts show all stages from early loculation to maturity.

The 10-day-old oocyst:—The average diameter of oocysts containing sporozoites (33 oocysts) is 60.6 (56–65). The salivary glands are positive for sporozoites.

On the 11th day only scanty oocysts were found and none were found on the 12th day. On both days the salivary glands were positive for sporozoites. On the 13th day the glands were negative and on the 14th day only 1 in 10 glands was positive. The overall rate for all dissected mosquitoes was 38%. Oocyst numbers per infected gut ranged from 1 to 150 (av. 23). The overall rate was affected by a microsporidian infection (Nosematidae) in about 30% of the *A. gambiae* used. This infection interfered with the development of the *Plasmodium* very considerably, causing excessive retardation of oocysts and apparently in some cases obliteration of the *Plasmodium* infection. The rate among microsporidia-free *A. gambiae* was 62%. For this reason all measurements given above refer to microsporidia-free *A. gambiae* only.

The number of sporozoites seen in any pair of salivary glands exceeded 50 on only 2 occasions. Generally the number seen was between 1 and 20, and the number seen bore no relation to the average number of oocysts seen, 23 per infected gut. Most of the live sporozoites seen were highly active, bending and snapping back to a straight shape with considerable speed at a temperature of 76°F. Warming to body temperature caused an activity comparable to that of coccidial sporozoites at the same temperature. On 2 occasions an infection of the glands was seen which might be called light as opposed to very scanty. Many sporozoites in these infections appeared to be dead. The average length of the sporozoites *in vivo* was 12.2 microns.

DISCUSSION

Some details of the sporogony of *P. schwetzi* have been described by Rodhain and Lassman (1940) and Rodhain (1955) in *A. atroparvus*. Rodhain and Lassman (1940) reported a 3% infection involving only 3 oocysts, 2 of which were very

large. Rodhain (1955) reported a rate of 66% in 2 lots of mosquitoes and among the oocysts seen were 6 which measured 70 to 74 microns in diameter on the 12th day after feeding. Sporozoites were reported in the salivary glands 14 days after feeding. The numbers of sporozoites found in the salivary glands were never large and the sporozoites disappeared quickly from the glands because they were expressed during biting, "or perhaps degenerated."

Rodhain (1955) states that the diameter of the mature oocysts of *P. schwetzi* is notably larger than that of similar stages of *P. vivax* and about the same as that of similar stages of *P. falciparum*. At present it is upon this statement only that *P. schwetzi* can be claimed to be a species distinct from *P. vivax*.

For this reason the present study has concentrated on the examination of the mature oocysts of *P. schwetzi*. No mature oocyst was found to exceed 65 microns in diameter if care was exercised to avoid compression of the cyst. The average diameter of the mature oocysts is 57.0 microns on the 9th day and 60.6 microns on the 10th day after feeding. Shute and Maryon (1952) give the diameter of the largest oocysts of *P. vivax* as 46 microns, but, as they fixed the oocysts in Bles solution, some shrinkage must be expected. In general, authorities agree that the diameter of the mature oocysts of *P. vivax* is between 45 microns and 55 microns. In this Institute oocysts of *P. vivax* in *A. gambiae* 10 days after feeding measure from 40 to 55 microns in diameter.[2]

Garnham (1955) has suggested that it would be useful to know in connection with Rodhain's demonstration (a) if cover-glasses had affected the size of the oocysts, and (b) what the pigment pattern was in the oocyst. From the present information it would seem possible that the oocysts seen by Rodhain were compressed, as there is at least 10 microns difference between his 6 large oocysts and the present series of 83 mature oocysts, and despite the possible strain differences.

The present figure of an average diameter of the mature oocyst of *P. schwetzi* (av. 57.0 and 60.6 microns, 47–65 microns) is undoubtedly a little larger than that generally reported for *P. vivax* of 50 microns with a range in our experience of 40–55 microns. However the ranges overlap by a considerable margin and, while this morphological difference might be taken to indicate a relative difference between variants, it certainly cannot be interpreted as an absolute difference between species.

The present study answers Garnham's second question. It can be seen from Figures 1 and 2 that the pigment patterns in the oocysts of *P. schwetzi* are indistinguishable from those of *P. vivax* at similar stages.

The fate of the sporozoites is interesting. The description of sporogony of *P. schwetzi* by Rodhain and Lassman (1940) led Bray (1954) to include this parasite in the growing list of plasmodia which complete oocystic development in certain mosquitoes but which fail to infect the glands with sporozoites. This has been borne out by the work of Rodhain (1955) and the present study. The number of sporozoites seen in glands is disproportionately lower than the number seen in oocysts and those glands which are infected apparently become negative quickly. Garnham (in press) has suggested that in such cases the sporozoites are killed by the haemocoelomic fluid of the mosquitoes.

In the present case the hyperactivity of the sporozoites while still in the in-

[2] I am indebted to Dr. R. M. Fox for these figures.

vertebrate host is interesting and may provide a clue to the killing action. If it is true that the naked sporozoites take in little nutriment from the invertebrate host such activity would quickly exhaust their slender metabolic stores. It may be that the killing action of the haemocoelomic fluid, at least in this case, is the excitation of hyperactivity in the sporozoite.

This evidence, despite successful laboratory transmission, and the failure of *P. reichenowi* to complete development in *A. gambiae* (Bray, in press) indicates that the transmission of chimpanzee malaria parasites is not effected in nature by *A. gambiae*.

In the light of our knowledge to date it can no longer be held that *P. schwetzi* represents a species distinct from *P. vivax*. This separation was made originally on the basis of host specificities but these have been shown since to be only relative. Bray (in press) and Garnham, Lainson and Gunders (personal communication) have shown that the splenectomized chimpanzee will harbor all forms of *P. vivax* in the same manner as man. Rodhain (1939) and Bray (in press) have shown that the whole chimpanzee will harbor an occult infection of *P. vivax* in the blood. Rodhain and Dellaert (1955) have shown that man will allow growth of *P. schwetzi* in the blood and that clinical symptoms (up to 11 paroxysms) ensue.

A. atroparvus is a well known vector of *P. vivax* and Bray (in press) has shown that *A. gambiae* is an efficient host for all sporogonous stages of *P. vivax*. Rodhain and Lassman (1940) and Rodhain (1955) have shown that *A. atroparvus* supports the growth of the oocysts of *P. schwetzi* but that the sporozoites do not fare well in this mosquito. The present study establishes a similar pattern of development for *P. schwetzi* in *A. gambiae*.

Morphologically the two parasites are identical except that the mature oocyst of *P. schwetzi* is a little larger than that of *P. vivax*. The differences are certainly insufficient to claim specific status for the chimpanzee parasite. On the other hand, the differences are sufficient at present to maintain that the 2 parasites are consistent variants. Therefore, it is proposed that the benign tertian malaria parasite of man be referred to as *Plasmodium vivax vivax*, when necessary, and the benign tertian malaria parasite of chimpanzees should be referred to as *Plasmodium vivax schwetzi*.

SUMMARY

A study of *Plasmodium vivax schwetzi* in *Anopheles gambiae* is described.

The sporogonous cycle occupies 10 days at 75°–80°F. The pigment pattern in the 4- and 5-day-old oocysts exactly resembles that of *P. vivax* at a similar stage. The mature oocysts have an average diameter of 57.0 microns on the 9th day and 60.6 microns on the 10 day after feeding.

There is an almost complete loss of sporozoites after release from the oocysts and the salivary glands of *A. gambiae* are very scantily infected. Despite this, transmission by sporozoites was successful. It is concluded that *A. gambiae* is not a suitable host or natural vector of *P. schwetzi*.

It is proposed that the benign tertian malaria parasite of man be referred to as *Plasmodium vivax vivax* when necessary, and the benign tertian malaria parasite of chimpanzees be referred to as *Plasmodium vivax schwetzi*.

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RESEARCH NOTE

THE OCCURRENCE OF *EPERYTHROZOON WENYONI*, *BARTONELLA BOVIS* AND *ANAPLASMA MARGINALE* IN AN OX IN KENYA

This note records the occurrence of *E. wenyoni* and *B. bovis* in Kenya for the first time.

During attempts to transmit tick-borne fever of cattle, blood was collected, into heparin, from 3 cattle showing tick-borne fever bodies in their leucocytes. This blood was pooled and inoculated subcutaneously into several cattle, including ox No. 2508. No parasites other than tick-borne fever bodies were seen in the limited number of blood smears available from the 3 donor animals.

Ox No. 2508 had a febrile reaction 10 days after inoculation and delicate filaments were seen, free in the plasma, in blood smears. Four days later typical forms of *E. wenyoni*, as described by Adler and Ellenbogen (1934, J. Comp. Path. and Therap. **47**: 219) were seen. These parasites persisted for 6 days. Annular forms were most common but filamentous and tennis-racquet forms were also frequent. Dumb-bell forms were rare. Most parasites were free in the plasma but some appeared to be attached to erythrocytes.

The same animal showed *B. bovis*, as described by Donatien and Lestoquard (1934, Bull. Soc. Path. exot. **27**: 652) in smears of peripheral blood 15 days after inoculation. These parasites persisted for 14 days, reached a maximum of 90 parasitized red cells per thousand, and disappeared 3 days before the animal died. Bacillary forms predominated but cocco-bacillary and coccoid forms were frequent. Up to 12 parasites were seen on a single red cell. Chains of parasites were common and all forms of the organism were seen in these chains which were made up of as many as 7 parasites. Most parasites appeared to be on the surface of erythrocytes but it could not be said with certainty that none was intracellular. The outline of the parasite was smooth, and no internal structure could be seen.

A. marginale appeared in blood smears 18 days after inoculation and persisted until the animal died 13 days later. *A. marginale* and *B. bovis* frequently parasitized the same erythrocyte. At post-mortem examination no lesions that could not be attributed to *A. marginale* were seen.

Nymphs of *Rhipicephalus appendiculatus* were allowed to feed on ox no. 2508 at a time when eperythrozoa were plentiful and bartonellae scanty, but failed to transmit these parasites when, after molting, they were allowed to feed on another ox.—D. W. BROCKLESBY, *East African Veterinary Research Organisation, Muguga, P.O. Box 32, Kikuyu, Kenya.*

HEPATOZOON GRISEISCIURI N. SP.; A NEW SPECIES OF *HEPATOZOON* FROM THE GREY SQUIRREL (*SCIURUS CAROLINENSIS* GMELIN, 1788), WITH STUDIES ON THE LIFE CYCLE*

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The protozoan genus *Hepatozoon* was first reported from a native animal in the eastern United States by Herman and Price, who in 1953 noted a high prevalence of gametocytes in the blood of grey squirrels at the Patuxent Research Refuge in Laurel, Maryland. This was the second time that this genus had been reported from a mammalian species indigenous to the nearctic region. The first such species, *Hepatozoon citellicola* (Wellman and Wherry, 1910) was found in *Citellus beecheyi* (Richardson, 1829), the California ground squirrel.

Previous to this, in 1908 Miller had noted and described *Hepatozoon perniciosum* (now *muris*) from laboratory white rats in Washington, D. C. In this paper Miller proposed the generic name and diagnosed genus and species by establishing both the schizogonic cycle in the liver of the rats and the sporogonic cycle in the mite *Echinolaelaps echidninus* (Berlese, 1887), which was clearly the arthropod vector.

The discovery of a species of *Hepatozoon* in the grey squirrel posed a problem in identification, especially since it was found in the same area as Miller's species. Nine references to *Hepatozoon* in the family Sciuridae are found in the literature. These are *H. citellicola* (Wellman and Wherry, 1910) from *Citellus beecheyi* in California; *H. mereschkowski* (Tartakovskii, 1913) from *Citellus suslicus guttatus* (Pallas, 1770) in Russia (Brumpt, 1946); *H. funambuli* (Patton, 1906) from *Funambulus pennanti* Wroughton, 1905 in India; *H. gaetulum* (Sergent, 1921) from *Atlantoxerus getulus* (Linnaeus, 1758) in North Africa; an undescribed *Hepatozoon* from *Petaurista petaurista* Pallas, 1776 in India (Wenyon, 1926); an undescribed *Hepatozoon* from *Meriones libycus erythrourus* (Gray, 1842) and *Spermophilopsis leptodactylus* (Lichtenstein, 1823) in the Murgab valley (Latyshev, 1949); *H. sciuri* (Coles, 1914) from *Sciurus vulgaris* Linnaeus, 1758 in England; *H. sciuri* (Franchini, 1932) from *Sciurus vulgaris* in Italy; an undescribed *Hepatozoon* from *Sciurus vulgaris* in China (Nauck, 1927); and the species dealt with in this paper. With the exception of *H. citellicola* whose large gametocytes (16×6 microns on an average) are significant, these squirrel-inhabiting forms are inadequately known for clear specific differentiation. Specific identity in *Hepatozoon* can not be clearly established until the various stages of the life cycle, both sexual and asexual, have been determined. Lacking this, the descriptions of most of the approximately 50 known species of *Hepatozoon* are inadequate. In order to establish clearly the identity of the grey squirrel *Hepatozoon*, work was initiated to determine the sexual cycle in natural and artificial arthropod vectors and the asexual cycle in the organs of infected squirrels.

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MATERIALS AND METHODS

In order to determine the possible vectors of this species in the local squirrel population, a qualitative survey of the ectoparasites of *Sciurus carolinensis* in the Washington–Maryland area was initiated. This survey included the parasites recovered from the nesting material of squirrel boxes in 4 areas. These areas were located first, at the western end of Washington Zoological Park; second, on the grounds of the Shephard-Pratt Hospital at Towson, Md.; third, in an extensive wooded area at Solomons, Md.; and fourth, at the Patuxent Research Refuge.

The parasites were collected from specially constructed nesting boxes at irregular intervals over a 2-year period by transferring the nesting material into paper sacks. These were brought into the laboratory and refrigerated at 36°–40° F. As soon as possible the material was emptied into a Tullgren-type separation apparatus which forced the nest fauna into water-filled collecting dishes.

All material from the separating funnel was checked under a stereomicroscope. Representatives of parasitic species were mounted and identified or collected alive and stored in a humidity chamber at 80% r.h. for further use.

Because of the variety of known arthropod vectors of *Hepatozoon*, it was decided to attempt to initiate sporogony in a wide range of arthropods. These included the mosquito *Aedes aegypti* (Linnaeus, 1762), the flea *Orchopeas howardii* (Baker, 1895), and the mites *Echinolaelaps echidninus* (Berlese, 1887), *Ornithonyssus bacoti* (Hirst, 1913), and *Euhaemogamasus ambulans* (Thorell, 1872).

Mosquitoes (*Aedes aegypti*), raised from eggs supplied from Orlando, Florida, were infected by placing an anesthetized, heavily infected squirrel into a feeding cage for approximately 30 minutes. When most of the mosquitoes had fed, the squirrel was removed. The mosquitoes thus infected were collected and held in small tubes at room temperature and 80% r.h. Mosquitoes were dissected in saline onto microscope slides at times ranging from 1 to 126 hours post infection. Three series of experiments were conducted involving approximately 50 mosquitoes in all.

Wild specimens of the flea *Orchopeas howardii* taken from nest boxes in the field were dissected in the laboratory. In addition, numbers of wild fleas were given constant access to a heavily infected squirrel in the laboratory by keeping them together in a large zinc culture can. Fleas were removed from this culture and examined at 1, 2, 3, and 4 weeks after being placed in the container.

Of the mites, *Echinolaelaps echidninus* was utilized extensively in the laboratory work on sporogony. While this species is not normally found on *Sciurus carolinensis*, there were a number of factors in favor of its use. First, it was felt that development of the grey squirrel *Hepatozoon* in *E. echidninus* would furnish an excellent diagnostic tool, since Miller had traced the cycle of *H. muris* in this mite. By comparing the development of two protozoan species in the same arthropod, significant differences could be evaluated. Secondly, *E. echidninus* is a large mite, easily cultured in the laboratory on rats. Hence, large numbers could be kept available. Finally, this mite feeds readily through silk bolting cloth and ingests a considerable amount of blood at each feeding.

In these experiments the mites were aspirated from the culture cans, 50 at a time, into glass vials (standard procain tubes), which consisted of 3-inch sections of 5/8-inch glass tubing. These vials were stoppered by fitting both ends with silk bolting cloth which was cemented to a small piece of quarter-inch rubber tubing. When inserted into the vial, these effectively restricted the mites, yet allowed a flow of air through the vial during aspiration. The mites held in such a cage could be artificially fed on infected blood through the bolting cloth by the Cross technique (Cross, 1954). This procedure standardized the time of infection and the relative amount of blood ingested, since fully fed mites could be readily distinguished through the glass by their bright red color and distended condition. The tubes were subsequently stored in a humidity chamber at about 80%–85% r.h.

Mites were removed from the tubes at intervals ranging from 10 minutes to 29 days after feeding. These were dissected into saline for observation in hanging drops, smeared by various methods to produce permanent slides or fixed in Susa fixative and later sectioned and stained with hematoxylin-eosin. If the mites were to be held alive longer than 7 days, it was found necessary to feed them on squirrel or rat blood every 3 to 4 days.

Tests with *Ornithonyssus bacoti* were conducted in the same manner. This species was checked for its ability to support sporogonic development by dissection into saline at times up to 92 hours after infection and by fixing and sectioning at times ranging from 1 hour to 8 days. *O. bacoti* was also maintained on an infected squirrel in the laboratory for 4 weeks. At the end of this time mites were removed from the culture and dissected.

Euhaemogamasus ambulans, a common inhabitant of squirrel nests in the field, was also utilized extensively in the tests. The life history of this species is much like that of the laelaptids. There is a small, fragile hexapod larva which has non-functional mouthparts and does not feed. The larva molts to become an octopod protonymph which is also rather colorless and fragile. Since the mouthparts are somewhat developed, this is probably a feeding stage. The protonymph molts to a well sclerotized, very active deutonymph which feeds actively. After feeding, the deutonymph molts to the adult male or female condition. Theoretically then, these mites could become infected at any time after the larval stage.

Wild *E. ambulans* from all areas visited were examined for the presence of *Hepatozoon* by dissection in the laboratory. Since a successful method of culturing *E. ambulans* in sufficient quantity for experimentation was not developed until late in the work, the species was not extensively used in the laboratory experiments.

In the course of the work a number of squirrels were routinely checked for *Hepatozoon*. The technique of tail bleeding was used for the most part. When a small piece was cut from the tip of the tail of an anesthetized squirrel, as much as 5 ml of blood could be collected in a short time. This method is especially good when repeated bleedings are necessary. Smears and sections of the internal organs of certain infected squirrels were made. In general, the liver, spleen and bone marrow were studied in this manner.

After some experimentation it was found that gametocytes could best be studied in the excysted motile condition by the following technique. About 2.5 ml of infected squirrel blood was placed in a standard centrifuge tube which was then filled to the 15-ml mark with distilled water. The blood and water were thoroughly mixed. After 3 minutes the tube was centrifuged at about 1500 r.p.m. for 10 to 15 minutes and the supernatant was carefully drawn off. The tube was then filled with physiological saline and the contents mixed. The tube was then recentrifuged and the supernatant drawn off leaving a residuum of motile gametocytes and blood cell remnants. This process served both to lake the blood cells, thus liberating the gametes, and to wash the remaining material in saline, which re-established a relatively normal osmotic pressure. The material thus treated was observed either as hanging drop preparations with phase contrast or as permanent slides by mixing, on a microscope slide, a small drop of the centrifuged material with a larger drop of clear squirrel blood serum, smearing, air-drying, and staining with Wright's stain.

Twenty-one young squirrels, ranging in age from 36 hours to 4 weeks, were examined for the presence of *Hepatozoon*. All but 2 of these were found to be positive on the basis of blood smears. In addition, tissue sections were made of the liver, spleen, bone marrow, brain, kidney and muscle of a female squirrel approximately 36 hours old. This animal was born in an apparently mite-free laboratory of an infected mother. Serial sections of whole squirrel embryos from an infected mother approximately 2.5 weeks post conception were also made.

RESULTS

The ectoparasite survey disclosed that the flea *O. howardii* and the mites *Euhaemogamasus ambulans* and *Haemolaelaps casalis* (Berlese, 1887) were common inhabitants of squirrel nesting material in Maryland. The populations of *O. howardii* and *E. ambulans* were surprisingly high when young were in the nest. The mites *Haemolaelaps glasgowi* (Ewing, 1925) and *Ornithonyssus bacoti* and the ticks *Ixodes marxi* Banks, 1908 and *Dermacentor variabilis* (Say, 1821) were occasionally found in the nest boxes.

Many infected monocytes could be found in the guts of infected mosquitoes shortly after the infecting blood meal. An hour after infection a number of excysted gametes were seen. These were distinctly vermiform in appearance and evidenced 2 types of movement. Occasionally they would glide, planaria-like, for short distances. More often they appeared to be attached to the substrate by one end which clearly is provided with a holdfast mechanism. With the narrow end holding and stationary, they contracted into a U, then sprang out again rapidly and repeatedly. At other times they were seen to lash in a spiral motion. No pairing activities of any kind were noted. Nothing that could definitely be identified as ookinetes or oocysts were identified in any of the mosquito preparations.

With the exception of a few questionable gametes, no forms were seen in any

preparations which suggested sporogonic development in either the flea *O. howardii* or the mite *O. bacoti.*

In the mite *E. echidninus,* the first forms found which had clear *Hepatozoon* characteristics were seen 7 days after the initial blood meal. These were circular sporonts which averaged about 52 microns in diameter (range, 23–69 microns). The surface of these spheres, which were rather resistant to stain, was regularly punctate. No nucleaus could be seen within the sporont (Fig. 1).

At 10 days post infection the sporonts measured from 80–102 microns in diameter and still showed the punctations quite clearly. In some cases, a distinct mottling of the sporont wall was noted. The oocyst wall appeared, in fresh preparations, as a clear envelope. Thirteen days after infection, measured oocysts ranged in diameter from 63–224 microns. Some of the larger cysts had a series of regularly spaced peripheral nuclei arranged on the oocyst wall (Fig. 2). These peripheral nuclei measured about 21 microns in diameter and took a light reddish stain.

By 20 days the sporonts had produced large numbers of daughter nuclei. In fresh preparations the sporont was seen almost to fill the oocyst, leaving a thin clear area. One sporont, which measured approximately 225 microns in diameter on a stained smear, contained over 200 daughter nuclei (Fig. 3).

After 28 days the oocysts usually rupture on dissection. Because of this the sporonts in stained smears appeared as irregular masses of developing sporoblasts. The sporoblasts at this time measure from 17–23 microns in diameter and stain a heavy, solid blue with Wright's stain. In fresh preparations the sporoblasts appear as delicate semi-opaque ovals with granular cytoplasm.

In the sections of *E. echidninus* fixed 29 days after the initial heavily infected blood meal, oocysts containing well formed sporozoites were seen (Fig. 4). The sporocysts were irregular and some of them had broken. Each sporocyst, as near as could be ascertained, contained but 4 sporozoites which were crescent-shaped bodies which measured from 11.8–13.2 microns in length. They were generally larger at one end, measuring about 1.9 microns at the small end and 2.6 microns at the large end. The nucleus was usually situated toward the smaller end. The largest mass of sporozoites seen covered an area 114×285 microns in the abdomen of the mite.

Many sporogonic forms were found in *Euhaemogamasus ambulans* collected from squirrel nests in the field. Excysted gametes in saline appeared similar to those from *E. echidninus.* In immature forms the spherical oocyst was filled with a clear fluid in which the sporont floated freely. The spherical sporonts were bounded by a tough cell membrane or pellicle. The cytoplasm was opaque and granular. A faint nuclear membrane could be discerned enclosing an extensive nucleus. The nucleoplasm was granular and appeared less dense than the cytoplasm. Within the nucleus was a prominent, dense karyosome. In many cases the karyosome contained a large, central vacuole or inclusion body and occasionally additional smaller ones could be seen (Fig. 5). In one typical cell the oocyst measured 140 microns in diameter, the sporont 85 microns in diameter, the nucleus 30 microns in diameter, and the karyosome 20 microns in diameter.

In more advanced forms the sporont was seen to have increased in size, filling a greater portion of the oocyst. As development proceeded, sporoblast nuclei could be seen on the periphery of the sporont. These gave rise to sporoblasts which increased in size during the formation of the sporozoites and, by the time the sporo-

cyst was formed, the mass of cells completely filled the oocyst (Fig. 6). Under phase contrast the sporocysts appeared as highly refractile ovals or spheres which regularly contained 4 sporozoites. The sporozoites, longitudinally oriented, completely filled the interior of the cyst. Mature sporocysts commonly ruptured during dissection, liberating the sporozoites. These were motile, banana-shaped cells, slightly larger at one end (Fig. 7). A refractile nucleus was located somewhat nearer to the small end. The sporozoites were slightly larger than motile gametes and measured about 25–30 × 5–6 microns. They were seen to glide slowly, wave sluggishly from side to side, and contract into an almost round form.

On stained smears oocysts were seen to contain as many as 200 sporoblasts which ranged from 4.6–6.6 microns in diameter. Maturing sporocysts were numerous in these smears. Early forms contained 2 nuclei which then divided into 4 (Fig. 8). As development progressed, these nuclei gave rise to sporozoites (Fig. 9).

Sections of naturally infected *E. ambulans* were found to contain sporogonic forms in all stages of development. Oocysts occurred in all body regions of the mites with the exception of the gnathosoma. The majority were located between or above the lateral gut diverticulae in the opisthosomal region. Occasionally cysts were found in the propodosomal region above or median to the anterior ends of the lateral gut diverticulae. Often the cysts were so extensive that they completely filled these areas, being as large as 206 × 57 microns.

In sections the oocysts do not appear as spherical, since they are molded to fit their environment (Fig. 17). In such cases only the sporont was measured. The average dimensions of the components of four typical cells were: sporont, 26 × 33 microns; nucleus, 15.5 × 13.5 microns; and karyosome, 7.7 microns in diameter.

Large clusters of sporoblasts, numbering as high as 200, could be seen in these sections (Figs. 10 and 17). The sporoblasts measured about 10.5 × 6.6 microns and had cytoplasm which took a dusky red stain.

Mature sporocysts measured 13.2 × 8.5 microns and contained the usual 4 longitudinally arranged sporozoites. Usually the 2 pairs of sporozoites were orientated in opposite directions which caused 2 nuclei to appear nearer one pole and 2 at the other.

Examination of adult squirrels showed that *Hepatozoon* is present in almost all the squirrels over an extensive area of eastern Maryland. Out of 24 adult squirrels bled, only 2 were negative. These animals, both females, were nesting in the attic of a private residence.

Gametocytes in fresh peripheral blood appeared as highly refractile, sausage-shaped bodies within monocytes. In fresh material the small end of the gametocyte was often seen to be recurved within the cyst. No movement of the gametocytes within the cysts was seen.

In stained slides the gametocytes are found to be encysted almost always in monocytes, although they are occasionally found free. The monocyte nucleus is often split into a few large masses. The gametocyte is enclosed in a clear, thin cyst which is more or less sausage-shaped and often appears slightly larger at one end. The average measurements of five representative forms are: cyst, 10.9 × 3.4 microns and nucleus, 4.7 × 3.4 microns. The tail end of the gametocyte was not generally recurved in stained smears. The nucleus is more or less central but may be closer to the small end. The nucleus is a rather oblong-shaped mass of reddish-blue staining material which may be discrete or at times show clear spaces within it.

The cytoplasm of the gametocyte stains a pale blue and contains a few reddish granules. Stained excysted gametocytes were crescent-shaped forms having average dimensions of 16.5 × 3 microns.

Smears and sections of the liver, spleen and bone marrow of infected adult squirrels failed to show schizogony. Smears and sections of the organs of 2 infected young about a month old also did not show schizogonic figures. It was not until sections and smears of a young squirrel only 36 hours old were made that schizogonic figures were seen. In the smear of femoral bone marrow made from this animal many schizogonic forms were evident. Some were elongate cells normally encysted in monocytes. These measured about 12.5 × 4.6 microns and had a nucleus arranged in transverse bands (Fig. 11). Other gametocytes were seen with nuclei which had split into a number of small masses (Fig. 12). The schizonts appeared in this preparation as characteristic ovals which were somewhat resistant to stain and took a light greenish blue with Giemsa (Fig. 13). A thin, clear cyst wall surrounded the central schizont. Many of these cysts appeared to be developing free in the marrow. The average measurements of 5 characteristic schizonts from the bone marrow were: schizocyst, 24.3 × 21.8 microns and schizont, 17.5 × 16.2 microns. No schizogonic forms were found in sections of the tail, intestine, brain, and kidney of this animal. Schizocysts were found in sections of the liver and the spleen. In both of these organs most of the schizocysts were found among the gland cells some distance away from blood vessels. Some of the cysts appeared to be free, while others showed an extremely flat nucleus on the periphery of the cyst (Fig. 14). Inside the schizocyst is a clear, empty space which indicates that the schizont has shrunk away from the cyst wall. Many schizonts showed numbers of small daughter nuclei (Fig. 15). Typical schizocysts in sections of the liver and spleen ranged in size from 16.5 × 13.2 microns to 23.1 × 18.4 microns; their schizonts from 11.8 × 11.8 microns to 18.4 × 13.2 microns. As many as 35 nuclei were counted. All of these cysts were approximately at the same stage of development. In addition, a second type of schizont was seen in the liver (Fig. 16). This form was distinctly smaller than the previous noted forms and contained but 4 large, fully developed merozoites. Cysts of this type measured about 15 × 8 microns.

No schizogonic forms could be distinguished in serial sections of the 2-week squirrel embryos.

DISCUSSION

The tests with *A. aegypti*, while not extensive enough to be conclusive, did strongly indicate that the squirrel *Hepatozoon* would not undergo sporogonic development in this mosquito. There was also no indication that the flea *O. howardii* or the mite *O. bacoti* would successfully support sporogony, although the large numbers of this flea in the squirrel nests made it highly suspect.

In the most important laboratory test with *E. echidninus* all of the mites with the exception of those examined during the first 7 days had been given more than 1 meal of infected squirrel blood. The time required for development by the parasites was reckoned from the time of the first feeding. While this affects the absolute accuracy of the results, it was reasoned that since the first meal contained the most gametocytes, it would be advisable to assume that the schizonts had consumed the maximum time in development. It is possible, therefore, that *Hepatozoon* of the grey squirrel can develop in *E. echidninus* in a shorter time.

The fact that all stages of the sporogonic cycle, including mature sporozoites, are found in *E. ambulans* taken from squirrel nests in the field strongly indicates that this mite is the natural vector. The high populations of this species which are attained at times and its common occurrence further emphasize this probability.

The diagnostic characters available from the sporogonic and schizogonic cycles rest largely on measurements. Unfortunately, such measurements are not sharp in their validity. There is a considerable variation between measurements of the same stage made under different conditions. In general, forms measured in the fresh condition are much larger than stained, smeared or sectioned forms. For example, the sporozoites of *Hepatozoon griseisciuri* measured approximately 12–13 × 2–2.5 microns in sections, 17–20 × 3 microns in smears, and 25–30 × 5–6 microns as live material. It is apparent that such measurements are not particularly valid for species comparison unless the exact conditions of measurement are considered.

The fact that most of the young suckling squirrels tested by bleeding were positive for *Hepatozoon*, often before their eyes were open, raised somewhat of a problem. It was known that the accepted prepatent time for the similar species *H. muris* was 12–14 days (Brumpt, 1946). Hence, assuming a similar prepatent time for *Hepatozoon* of the grey squirrel, some of the baby squirrels would have had to be infected at, or shortly after, birth. Assuming the oral route is necessary for infection, the young squirrels, while very young and first beginning to suck, must have ingested infected arthropods. When finally a full blown infection was found in smears and sections of a squirrel born in a mite-free environment at a known age of about 36 hours, it strongly indicated that the infection was acquired before birth, presumably across the placenta.

A number of problems associated with the schizogonic cycle remain unanswered. Considering that schizogony was found in the liver, spleen, and bone marrow and considering what is known of other species, it seems possible that schizogony in the rodent-inhabiting species of *Hepatozoon* occurs throughout the blood spaces of the reticulo-endothelial system without special emphasis on a particular organ. The schizonts may occur free and are surrounded by a definite schizocyst.

Hepatozoon griseisciuri n. sp.

Diagnosis: Encysted gametocytes in fresh material approximately 12 × 4 microns in size, on stained slides ranging from 9.9–11.8 microns in length by 3.3–4 microns in width. Gametocyte nucleus on stained slides ranging from 4 × 3.3 to 5.2 × 3.3 microns. Excysted gametes on stained slides measuring from 22–25 microns in length by 2–2.5 microns in width. Gametes in fresh preparations motile, approximately 25 × 2–3 microns in size. Sporogonic development possible in the mites *Euhaemogamasus ambulans* and *Echinolaelaps echidninus*. Mature oocysts in stained sections ranging from 200 to 285 microns in diameter and containing 200 or more sporocysts. Sporocysts in stained sections measuring about 13.2 × 8.5 microns, on stained smears 23–24 × 13–14 microns, and 25–30 × 20–25 microns in fresh material. Usually with 4 banana-shaped sporozoites in each sporocyst. Sporozoites in stained sections measuring approximately 11.8–13.2 microns in length by 1.9–2.6 microns in width, in stained smears about 17–20 × 3 microns, and 25–30 × 5–6 microns in fresh material. Schizogony occurring in the bone marrow, liver, and spleen of *Sciurus carolinensis*. With 2 types of schizocysts; small ones containing about 4 large merozoites and large ones containing as many as 35 daughter nuclei. Large schizocysts in stained smears ranging in size from 19.1 × 21.7 microns to 29 × 25.7 microns with schizonts ranging from 16.5 × 14.5 microns to 21.7 × 15.1 microns. In stained sections large schizocysts ranging from 16.5 × 20 microns to 23.1 × 18.4 microns in size with schizonts ranging from 13.2 × 16.5 microns to 18.4 × 13.2 microns in size.

Hepatozoon griseisciuri may be distinguished from all other adequately known species of *Hepatozoon* by the fact that the sporocysts regularly contain only 4 large sporozoites.

Type specimen. The holotype, a section slide of *Euhaemogamasus ambulans* containing an immature oocyst illustrating the karyosome, a group of sporoblasts, and a group of sporocysts, is deposited in the collection of the United States National Museum, Washington, D. C., under the number 23517. The large cluster of sporocysts between sporoblasts and immature oocyst is strictly designated as the type (Fig. 17).

Type host: Sciurus carolinensis, the eastern grey squirrel.

Type locality: Eastern Maryland.

SUMMARY

Hepatozoon griseisciuri n. sp. is described from *Sciurus carolinensis,* the eastern grey squirrel. Sporogony in the mites *Echinolaelaps echidninus* in the laboratory and *Euhaemogamasus ambulans* from squirrel nests in the field is reported. Schizogony in the spleen, liver, and bone marrow of a squirrel 36 hours old is described.

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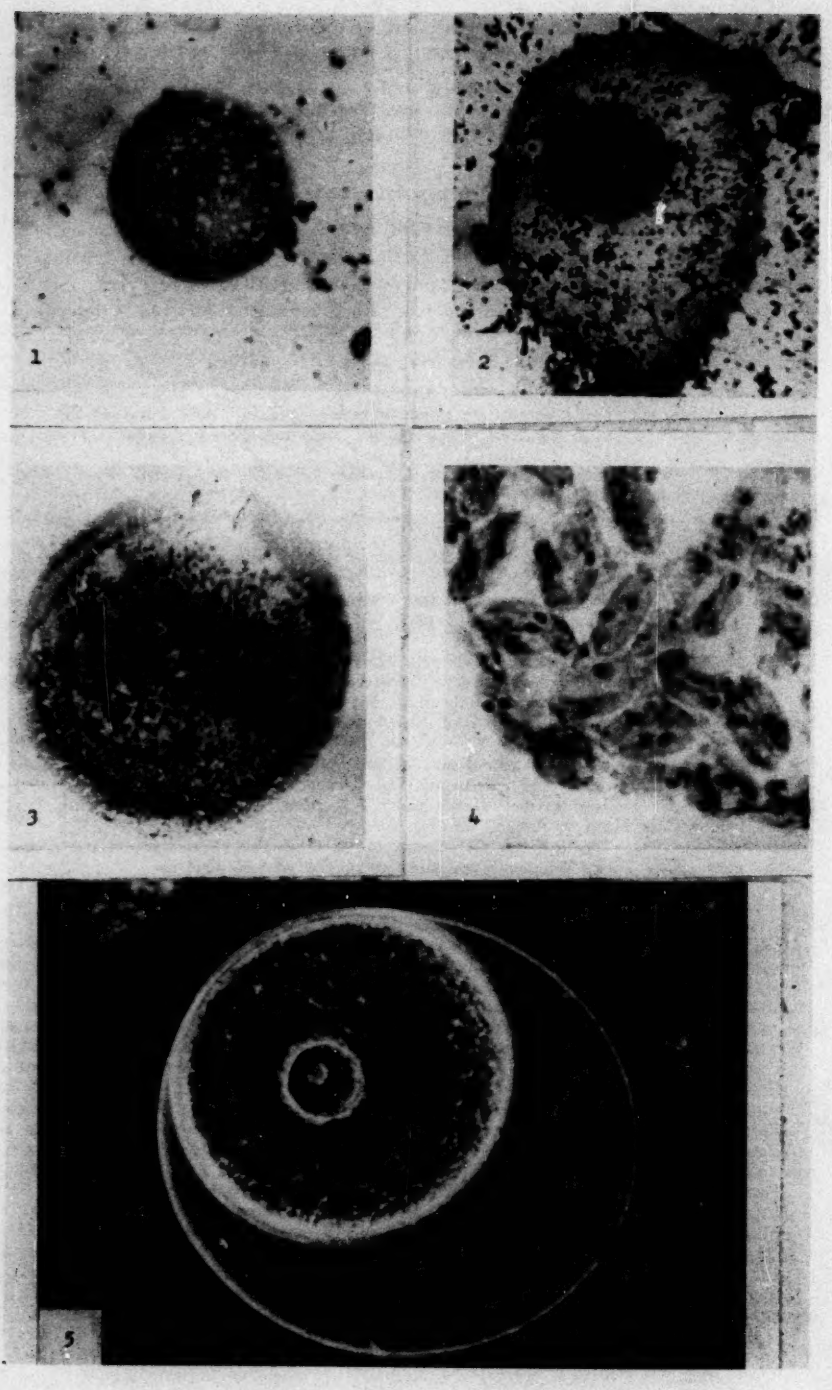
Explanation of Plates

Figure 1. Sporont from stained smear of *E. echidninus* 7 days after ingestion.
Figure 2. Oocyst from stained smear of *E. echidninus* 13 days after ingestion.
Figure 3. Sporont from stained smear of *E. echidninus* 20 days after infection.
Figure 4. Sporozoites from stained section of *E. echidninus* 29 days after infection.
Figure 5. Living oocyst in saline smear of *E. ambulans* under phase contrast.
Figure 6. Living, mature oocyst in saline smear of *E. ambulans* under phase contrast.
Figure 7. Living sporozoites in saline smear of *E. ambulans* under phase contrast.
Figure 8. Sporocyst in stained smear of *E. ambulans.*
Figure 9. Mature sporocyst in stained smear of *E. ambulans.*
Figure 10. Sporoblasts in stained section of *E. ambulans.*
Figure 11. Gametocyte in stained smear of the femoral bone marrow of a squirrel 36 hours old.
Figure 12. Early schizont in stained smear of the femoral bone marrow.
Figure 13. Developing schizont in stained smear of femoral bone marrow.
Figure 14. Schizont in stained section of the spleen of a squirrel 36 hours old.
Figure 15. Actively dividing schizont in stained section of liver.
Figure 16. Section of liver showing small schizocyst and 2 merozoites.
Figure 17. Holotype section of *E. ambulans* showing, from lower left to right, a cluster of sporoblasts, a cluster of sporocysts, and a developing oocyst.

PLATE I



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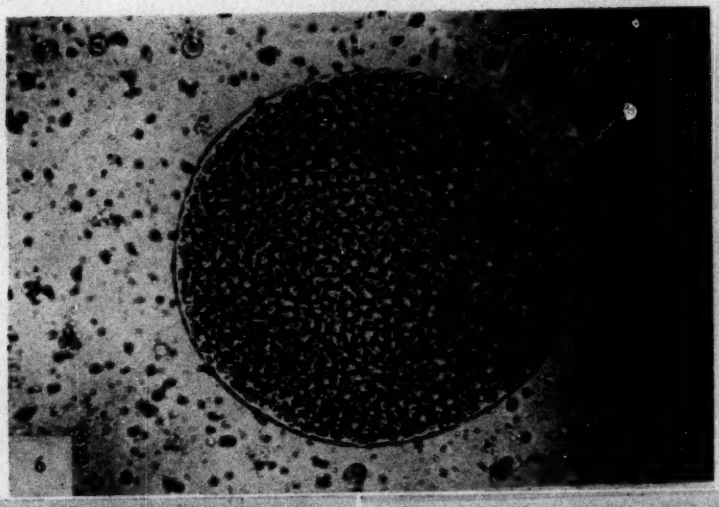
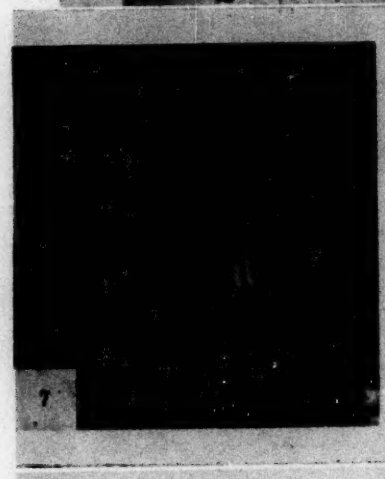
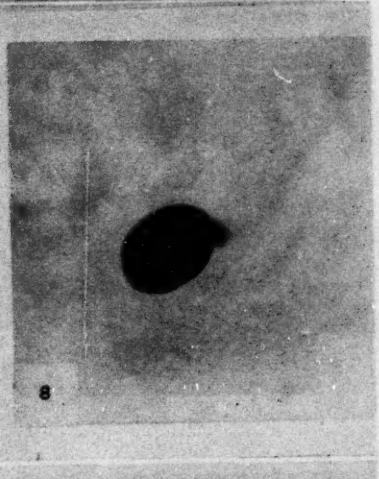






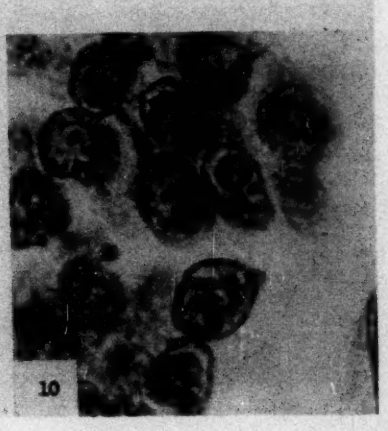


PLATE III

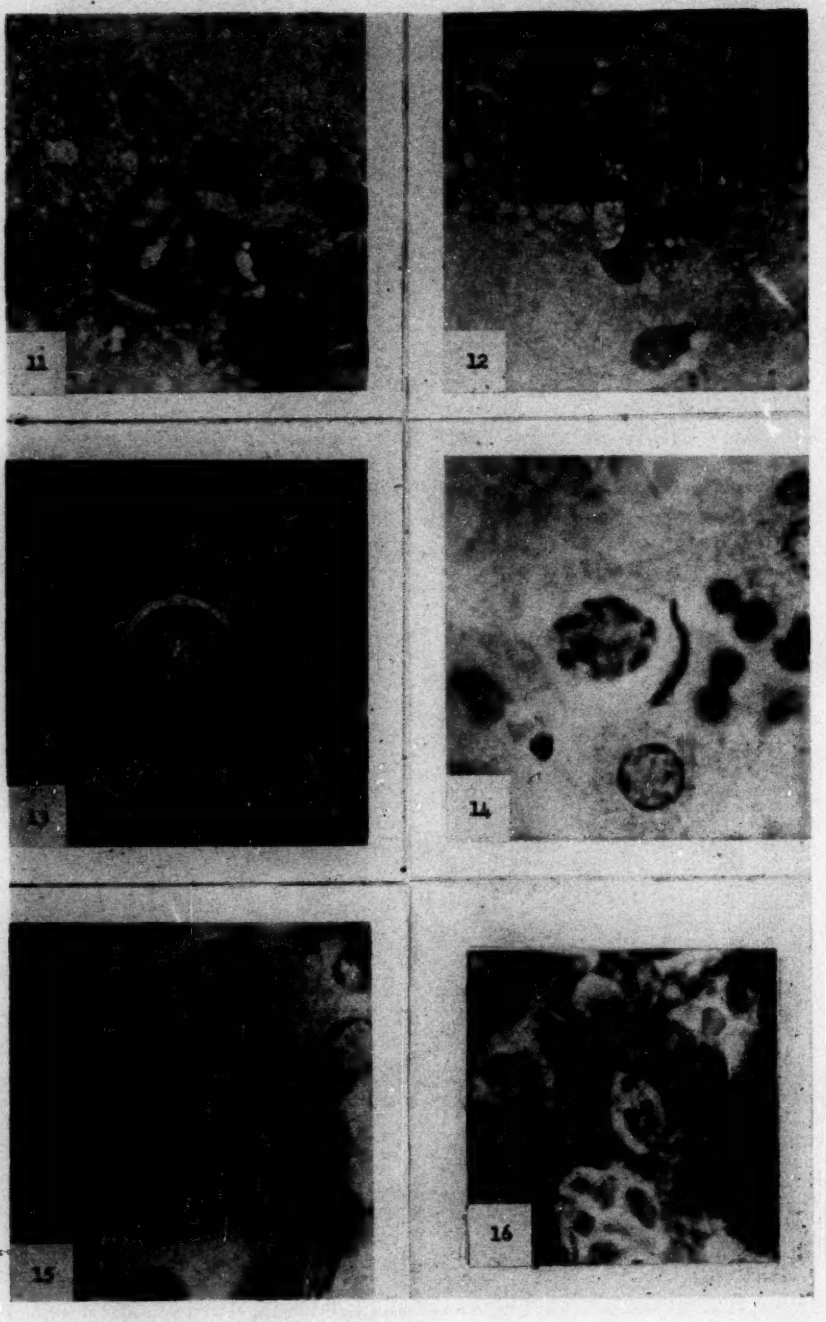
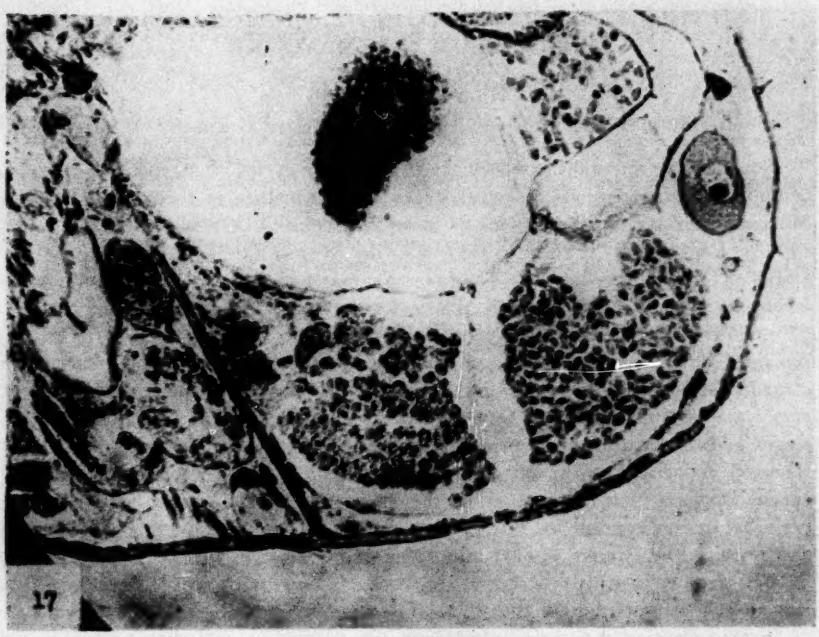


PLATE IV



RESEARCH NOTE

METAGONIMOIDES OREGONENSIS PRICE, 1931 FROM A GEORGIA RACCOON WITH A NOTE ON *SELLACOTYLE MUSTELAE* WALLACE, 1935

During a rabies study in Newton, Georgia, Dr. Denny Constantine, Communicable Disease Center, Public Health Service, noted that a captive raccoon, *Procyon lotor*, had frequent, tarry, fluid stools. The animal died and was referred to the author for parasitological examination. The following worms were recovered from the intestines: *Oochoristica procyonis* 11, *Molineus barbatus*, 93, *Physaloptera rara* 20, *Fibricola texensis* 33, *Pharyngostomoides procyonis* 29, *Eurytrema procyonis* 5, *Sellacotyle mustelae* 11, *Metagonimoides oregonensis* 12, a total of 214 worms. In addition, several specimens of bat trematodes were found; these were in rather poor condition and it is doubted that the parasites had become established in the raccoon. The animal had been fed 2 species of bats, *Nycticeius humeralis* and *Tadarida cynocepala*, during the rabies study. *S. mustelae* was reported from the mink in northern U. S. and from the raccoon experimentally by Wallace (1935, J. Parasitol. **21**: 143–164).

The presence of *M. oregonensis* in this animal is of particular interest. This trematode was first described by Price (1931, J. Wash. Acad. Sci. **21**: 405–407) from specimens collected in Corvallis, Oregon. A review of the literature failed to reveal any reports of this parasite since the original description. The present report extends the geographic range of *M. oregonensis* to include Georgia and establishes the raccoon to be a natural host of *S. mustelae* in Georgia.

The author is indebted to Dr. Emmet W. Price and Mr. Allen McIntosh for verification of the identification of *M. oregonensis*, and to Dr. Asa Chandler for his examination of the bat trematodes.—THOMAS K. SAWYER, *Laboratory of Rickettsial Biology, Walter Reed Army Institute of Research, Washington, D. C.*

OBSERVATIONS ON THE DEVELOPMENT OF THE MIRACIDIUM AND THE GERM CELL CYCLE IN *HERONIMUS CHELYDRAE* MacCALLUM (TREMATODA)

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Considerable attention has been given to the life cycles of digenetic trematodes because of the successions of larval stages and the nature of their multiplication. Morphological investigations, such as those of Brooks (1930), Cable (1934), Chen (1937), Rees (1940), Peiper (1953), Van der Woude (1954), and the numerous works of Cort and his coworkers, support a concept of germinal lineage with polyembryony to explain the multiplication and succession. According to this concept, germinal cells in the miracidium-mother sporocyst, secondary sporocysts, or rediae, remain distinct from the soma and give rise by mitotic division to the successive stage in the life cycle. Woodhead (1931, 1954, 1955 and 1957) presented a concept of polymorphism, whereby he believed that in several species of trematodes, sexual stages and fertilization occur prior to the successive larval stage. Ciordia (1956) repeated Woodhead's work on *Rhipidocotyle papillosum* and found no evidence of sexual stages or of fertilization in the sporocysts. His results supported the germinal lineage concept for this species. Other earlier theories were summarized and reviewed by Brooks (1930) and Cable (1934).

The manner in which the larval stages of *Heronimus chelydrae* develop makes this an excellent species for the study of a germ cell cycle. A complete sequence of developmental stages of the miracidium occurs within membranous shells in the long uterus of the adult worm. Miracidia containing germinal cells and young cercarial embryos are expelled from the adult as large fully developed forms described by Lynch (1934). Because of the membranous nature of the shell, sections from the mature worm with all developmental stages of the miracidium can be observed, well fixed and without interference from cracked shell particles. Early in the summer of 1956, snails were infected with the miracidia of *H. chelydrae* and mother sporocysts with developing cercariae became available for study. This development permitted the tracing of a germ cell cycle from the 2-cell stage of the miracidium to the genital primordium of the cercaria. Both R. M. Cable and R. B. Crandall at Purdue University, and M. J. Ulmer at Iowa State College are working on other phases of the life cycle of *H. chelydrae* at present. Cable and Crandall (1946) have placed the genus *Heronimus* in the superfamily Paramphistomatoidea because of the structure of the cercaria which was found to be similar to those of other species in this superfamily. A study of gametogenesis in the adult form of *H. chelydrae* by Guilford (1955) showed that maturation of sperm and ova, and fertilization in the mature form of this species are essentially the same as in other species of digenetic trematodes.

Previous comprehensive studies using sections of the developmental stages of the miracidia of *Fasciola hepatica, Fasciolopsis buski, Paragonimus kellicotti, Parorchis acanthus,* and *Megalodiscus temperatus* have been made respectively by

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Ortmann (1908), Ishii (1934), Chen (1937), Rees (1940), and Van der Woude (1954). The development of the miracidium of the amphistome *Cotylophoron cotylophorum* also was studied in considerable detail by Bennett (1936).

MATERIALS AND METHODS

Living specimens of *H. chelydrae* were collected from the lungs of *Chrysemys picta*. The developmental stages of the miracidia were studied mainly from serial sections, 6 to 20 microns thick, from worms which had been fixed without pressure in Bouin's Fluid or AFA. These were stained with iron hematoxylin or with Mallory's Triple Stain. Living stages were studied in eggs teased from the uterus of the adult worm in normal saline. Miracidia recently hatched from such eggs were transferred from normal saline to fresh water and used to infect laboratory raised snails of the genus *Physa*. Mother sporocysts and cercariae dissected from the body cavity of the snails were used for observations while living or after fixation and staining. The bodies of infected snails from which shells had been removed were fixed in AFA or Bouin's Fluid and were sectioned serially for the study of the germinal cells in the sporocyst and for the study of the genital primordium of the cercaria. All measurements and drawings were made from fixed or sectioned materials. Drawings were made with the aid of a camera lucida.

OBSERVATIONS AND DISCUSSION

Early development of the miracidium: A differentiation of cells can be observed very early in the development of the miracidium of *Heronimus*. The 2-cell stage of the miracidium consists of a macromere and a micromere which result from an unequal division of the zygote. The division is mitotic (Guilford, 1955). Of 20 2-cell stages measured, the macromeres varied in diameter from 18 to 30 microns with an average of 25 microns while the micromeres varied from 15 to 25 microns with an average diameter of 17 microns. The diameter of the macromere nuclei varied from 10 to 15 microns, while those of the micromeres varied from 10 to 12 microns. The nuclei of both cells have similar staining reactions, and each may possess 1 or 2 nucleoli. The macromere divides first producing a stage with 3 approximately equal sized cells of similar appearance (Figs. 1 and 2). Because of the similarity in cell size and appearance, the micromere is difficult to identify in this stage unless, as in Figure 3, the derivatives of the macromere have mitotic figures and the micromere is in an interphase. To form the 5- to 8-cell stages, cells at the anterior end of the embryo continue to divide while the micromere at the posterior end remains undivided and thus is proportionately larger with a proportionately larger nucleus and nucleolus (Figs. 4 and 5). In the 8- to 10-cell stages this latter cell divides slightly unequally to form 2 cells which have larger nuclei and nucleoli than the other cells of the embryo (Fig. 6).

In almost every trematode in which the first cleavage division has been described, it has been an unequal one. Recent papers on other trematodes use the terminology introduced by Ishii (1934), and refer to the macromere as an "ectodermal cell" and to the micromere, which may possess a dark chromatic nucleus, as a "propagatory cell." Ishii (1934), Chen (1937), Rees (1940), and Van der Woude (1954) have traced the origins of the germinal cells to the propagatory cell while the somatic origins were traced to derivatives of both ectodermal and propagatory cells depending on the form. Chen and Van der Woude also described differences in the very early descendents of the ectodermal cells. These were not observed in *Heronimus*. Whereas in other forms it is distinct, the micromere in *Heronimus* is difficult to distinguish in the 3-cell stage. Development preceding and following this stage, however, is similar to that in other trematodes so far described. It then appears

that, with minor differences, the early stages of the development of the miracidium of *Heronimus* are homologous to the other digenea, and that the posterior cell of the 5- to 8-cell stages is a "propagatory cell" and that the anterior mass of cells is derived from the macromere or "ectodermal cell". Willey and Godman (1951), in the paramphistome, *Zygocotyle lunata*, found an uneven first cleavage and traced the large cell at the end of the embryo through the 5-cell stage, but they were unable to confirm that this large cell was an early segregation of the germinal propagatory cell.

Formation of vitelline membrane cells: Vitelline membrane cells varying in diameter from 3 to 6 microns and possessing clear nuclei, each with a proportionately large nucleolus, appear in various places along the periphery of the early embryo (Figs. 8 and 9). Between the 8- and 30-cell stage of the miracidium a succession of these cells is formed by division of ectodermal cell derivatives. A similar origin of vitelline membrane cells was described by Schubmann (1905), Ortmann (1908), Ishii (1934), Chen (1937), Rees (1940), and Van der Woude (1954). These authors found that entire cells leave the surface of the embryo, migrate towards the shell, and fuse to form a membrane. In *Heronimus*, however, the plasma membrane of these cells disintegrates and the free nuclei leave the surface of the embryo and circulate among the vitelline cells in the egg and around the young embryo. Eventually most of the nuclei take a peripheral position adjacent to the shell. By the time these nuclei migrate, the vitelline cells have undergone degenerative changes in which the nuclear membrane breaks down and the vitelline cells appear as dark staining masses. By the time the embryo has reached a 40- to 50-cell stage the vitelline cells have completely disintegrated and a mucoid material from them is found around the periphery of the shell. In almost every case the free nuclei of the vitelline membrane cells are found in this material. Between this mucoid material and the embryo there is a clear sphere of fluid in which an occasional vitelline membrane nucleus is found. Although already enlarged to diameters of up to 15 to 30 microns by the time they reach the shell periphery, the vitelline membrane nuclei continue to enlarge and become flattened disc-shaped structures of varying degrees of thickness and diameter depending upon the position within the shell and the pressure upon them. The nucleoli within enlarge to diameters of from 7 to 9 microns and in later stages they appear vacuolated. Figure 9 shows one of the vitelline membrane nuclei next to the periphery of the shell. Throughout most of the development of the miracidium this mucoid membrane surrounds the serous material in which the embryo develops, but as the miracidium reaches its fullest development, the nuclei of the vitelline membrane cells may disintegrate as the mucoid material is pushed into a mass and forced by movement of the miracidium into varying locations within the shell.

The shell of *Heronimus* also undergoes changes and stretches from about 45 microns in length just after formation to a maximum of from 200 to 300 microns at which time it accommodates the fully grown miracidium. Changes in the staining reactions of the shell are obvious when specimens are stained with Mallory's Triple Stain. When the embryo is small the shell shows bright red vitelline droplets. As the embryo grows and the shell stretches, the red appearance is replaced by a thin blue-staining homogeneous shell. If an oocyte, zygote, or young embryo does not develop, the shell retains its original size and appearance as it

travels through the uterus. Although vitelline cells within these eggs may become pyknotic, they do not disintegrate to form a mucoid membrane.

Formation of Epidermis and subepidermis: Epidermal cell nuclei are distinguished first projecting slightly from the periphery of the 40- to 50-cell stages of the embryo (Fig. 10). Each nucleus possesses a single nucleolus. The cell boundaries of the epidermal cells are indistinct at this time. As the embryo enlarges, slightly spindle-shaped protoplasts without cilia become evident around the epidermal nuclei (Fig. 12). By the time the embryo reaches a length of 90 microns the epidermal cells become more spindle-shaped and borders contact one another (Fig. 13). Short cilia may be present at the posterior ends of the cells. By the time the embryo is 120 microns in length and is in the shape of the mature miracidium, cilia cover the outer boundaries of the epidermal cells. (Fig. 14). These cells continue to grow to the thin flattened structures with long cilia described by Lynch (1934). Small round cells, possibly the primordia of simple receptors were noted between tiers of the epidermal cells by the 120-micron stage of the miracidium. The epidermal cells remain on the miracidium as it penetrates the snail but are lost as the sporocyst develops.

As early as the 100-cell embryo the small round nuclei of the primordial subepithelium become evident (Fig. 11). These cells are located along the periphery of the embryo just within the layer of epidermal cells. These primordial cells develop into the uni- and binucleate cells described by Lynch (1934) and they remain with some further divisions, in the sporocyst.

Development of glands: Lynch (1934) described 3 types of glands in the miracidium of *Heronimus:* A single apical gland with 4 nuclei at its base, a pair of large lateral cephalic glands, and an anterior pair of small oxyphilic glands.

In the development of the miracidium, the primordia of the apical glands appear as 4 nuclei in the anterior median region of the 60-micron embryo (Fig. 12). As the embryo increases in length, the nuclei of these glands enlarge and are surrounded by reticulate cytoplasts with distinct cell boundaries (Fig. 14). Eventually the 4 cells fuse and remain fused as in the fully developed gland. Also in the 60-micron embryo, cephalic gland primordia are present on the lateral sides as 2 large nuclei each possessing a large nucleolus (Fig. 12). Later these nuclei become surrounded by a reticulated cytoplast and become the largest cells of the developing miracidium. By the 90-micron embryo, oxyphilic cell primordia appear dorsally to the median apical gland cell, and these elongate as the miracidium grows (Fig. 14). The glands disintegrate as the sporocyst develops.

Development of the nervous system: Nerve cell primordia, in the position of the future brain become evident as a group of small cells with lightly staining nuclei by the time the embryo reaches a length of 50 microns. Between the 90- and 120-micron embryo protoplasmic axons and dendrites can be found projecting from these cells (Figs. 13 and 14). By the time the embryo is 140 microns in length the central nerve mass is well developed. Dorsal to the nerve mass, and on each side of the 80-micron embryo, 2 hemispherical cells adjacent to one another, the primordia of eyespots, can be found. Pigment granules begin to appear in these cells after the embryo reaches a length of 150 microns. The pigmented eye spots remain throughout the life of the mother sporocyst.

Somatic structures in the body cavity: The body cavity begins to develop at the pos-

terior end of the embryo when it is about 50 microns long (Fig. 11). By the time the embryo is 60 microns long, parenchymal cells are evident within it. Some of the parenchymal cells develop into supporting structures while others develop into the cells of the excretory tubules between the 80- and 90-micron embryo. One lateral and 1 posterior flame cell can be found at this time. Flame cells and excretory tubules are retained within the sporocyst.

The sequence of somatic development in *H. chelydrae* is similar to that of other miracidia in which development has been described, in that vitelline membrane cells develop first, epidermal and subepithelial cells secondly, while nerve and excretory structures are evident later in development. With the exception of the vitelline membrane cells which appear as vestigial structures in *H. chelydrae*, the somatic tissues develop in a manner similar to those of other miracidia described. In *H. chelydrae* the vitelline membrane cells, epidermal cells, subepithelium, nervous system and glands all appear to arise from the anterior mass of cells that were derivatives of the ectodermal cell, while the light-staining parenchymal cells and excretory structures in the body cavity are possibly derivatives of the propagatory cell. Although Van der Woude (1954) believed that gland, parenchyma, and excretory structures might be derivatives of the propagatory cell, and Ishii (1934) and Chen (1937) found that the propagatory cell gave rise to muscle and glands, there is a general agreement that most of the somatic structures are derivatives of the ectodermal cell.

Germinal cell development: Lynch (1934) described a mass of "germ balls" located in the body cavity at the posterior end of the fully developed miracidium. He found that each individual germ ball was enclosed in a protoplasmic membrane which was attached directly or indirectly to the outer wall of the body cavity, and that the posterior end of the entire mass formed an appendage which turned dorsally against the body wall. He described the germ balls as multicellular or at least multinucleate structures of varying sizes with from as few as 2 or 3 cells to more complex structures 35 to 41 microns in diameter, the latter with cavities.

In observations on miracidial development it was found that the primordium of the germinal mass can be distinguished when the body cavity has just become evident within the embryo. This mass, composed of large light-staining germinal and parenchymal cell primordia, can be found on the dorsal side of the body cavity (Fig. 11). From their location, these cells appear to have arisen from the large dark-staining derivatives of the propagatory cells found at the posterior end of the 10- to 50-cell stages of the miracidial embryo (Figs. 7, 8, and 10). As the miracidium develops the primordium grows into the body cavity from the dorsal posterior side (Figs. 12, 13, and 14) and attains a length of 60 to 70 microns by the time the embryo reaches a length of 140 to 150 microns (Fig. 15). The smallest nuclei of this mass are dark-staining, round or oval, and are about 3 to 4 microns in diameter; these may be paired, presumably just after a nuclear division. They are found at the proximal end of the mass. These nuclei increase in size and become either the spherical or oval primordia of the germinal cells 6 to 7 microns in diameter, or the lighter-staining and smaller primordial parenchyma cell nuclei, 4 to 5 microns in diameter. The few divisions observed in the formation of these cells were mitotic. The cytoplasmic boundaries of these cells are indistinct in the proximal regions of the mass. Fully developed germinal cells, such as have been

described in many other species of trematodes, differentiate at the more distal end of the mass. Development of the primordial cells into fully developed germinal cells is characterized by a further increase in the size of the nucleus, in which the chromatin becomes evenly distributed accounting for a lighter-staining nucleus. There is also an increase in the size of the nucleolus, and the cytoplasmic boundaries of the cells become defined. At the end of their growth period, these cells are 10 to 12 microns in diameter with nuclei 7 to 9 microns in diameter with large nucleoli. These large fully developed germinal cells in the anterior region of the mass separate while the smaller parenchyma cells in the same region differentiate and produce a network of protoplasmic processes between the germinal cells (Fig. 16). Some of the processes of these cells connect with the parenchymal cells on the outer periphery of the body cavity and support the germinal mass.

Nuclear division in the germinal cells was carefully studied and in favorable sections the chromosomes were counted. In the later prophase or metaphase where individual chromosomes were distinct, a diploid number of 20 chromosomes was counted (Fig. 17). Anaphases of the first division are shown in Figures 18 and 19. Upon division these cells develop into cercarial embryos.

Development of the cercaria: The development of cercarial embryos from germinal cells was studied in both the miracidium and the mother sporocyst. The latter develops from the miracidium after its penetration into the intermediate host. Since Crandell and Cable, and Ulmer are working on other phases of the life cycle, only the early development of the cercarial embryo from the germinal cells and the development of the genital primordium were studied.

Germinal cells at the distal end of the mass produce new cercarial embryos, while primordial cells at the proximal end of the mass differentiate into more germinal cells. As new cercarial embryos are formed the number of new germinal cells is reduced, thus limiting the number of cercarial embryos. In the 4-day sporocyst both new germinal cells and embryos are found in the germinal mass. The arrangement of the embryos and germinal cells is still similar to the arrangement in the miracidium, and the embryos still are enveloped within the parenchymous network (Fig. 30). Within the 12-day sporocyst, after the embryos have increased in size and the enveloping parenchymatous network no longer supports them, the embryos move freely back and forth with contractions in the body wall. In this stage there are still a few germinal cells in the posterior end of the sporocyst (Fig. 31). After 15 days of development in the snail, cercarial embryos are shed into the body cavity to complete their development.

The first division of the germinal cells is unequal and results in a 2-cell cercarial embryo with 1 macromere and 1 micromere (Fig. 20). In some stages where there is a very small micromere nucleus, the micromere may appear to be like a polar body, but since the division of the germinal cells is mitotic, the resemblance is only a superficial one. After the first cleavage division, the micromere nucleus divides several times to form 2 to 6 nuclei adjacent to the macromere nucleus (Figs. 20–21). Following this the macromere nucleus divides into 2 large nuclei; thus the 8-cell stage of a cercaria is composed of 2 distinct macromere nuclei and 6 micromere nuclei (Fig. 22). By the 16-cell stage both macromere and micromere nuclei have divided again and the embryo consists of 4 macromeres and 12 micromeres (Fig. 23). Two investing cells, developed from macromeres at one side of the cercarial

embryo, are distinguishable as early as the 27-cell stage (Fig. 24). From the 27-cell stage until there is some differentiation of primordial cells of the cercaria, the young embryo appears as a complicated mass of macromeres and micromeres. The macromeres usually are located in the central portion of the young embryos while the micromeres generally take a peripheral position.

The genital primordium of the cercaria first can be distinguished as a medially placed mass of intermediate sized nuclei when the embryo is about 50 microns long (Fig. 25). It was not determined whether the cells in the genital primordium were descendants of the macromeres or micromeres but their location would suggest development from the macromeres. This primordium projects into the body cavity of the cercaria and remains distinct from the somatic tissues. It increases in size until the cercaria is fully developed (Figs. 26, 27, and 28). In the fully developed amphistome cercaria the genital primordium is a compact mass of deeply staining cells located in the body cavity just anterior and dorsal to the posterior sucker (Fig. 29). This mass is typical of the genital primordia of other types of cercaria in that the cells can be distinguished from the surrounding soma by their sizes, position, and staining reactions. The cells in the mass have a small amount of cytoplasm, indistinct cell boundaries and possess dark-staining round or oval nuclei.

In the author's previous study (1955) of the mature form of *H. chelydrae*, spermatogenesis, oogenesis, and fertilization with the formation of male and female pronuclei, were described. These stages in the mature form were similar to homologous stages in other species of digenetic trematodes. In the present study, observations on the germinal cells within the miracidium-mother sporocyst show that the fully developed germinal cells have a diploid number of chromosomes, divide by mitosis, and develop into cercarial embryos. Meiotic divisions, polar body formation, and fertilization stages similar to those in the mature form were not observed in the miracidium-mother sporocysts, nor were any stages found that were interpreted as cells undergoing meiosis or fertilization such as described by Woodhead (1931, 1954, 1955, and 1957) in other species of trematodes. The observations on the germ cell cycle in *H. chelydrae* are in agreement with the previous complete accounts of germ cell cycles by Chen (1937), Rees (1940), Peiper (1953), and Van de Woude (1954), which traced a direct line of germinal cells from the fertilized egg of the mature form to the genital primordium of the cercaria and consequently to the ovary and testes of the mature form. In their accounts there is more than 1 generation of segregated germinal cells since there are secondary germinal sacs in the form of secondary sporocysts or rediae. In *H. chelydrae* the life cycle and consequently the germ cell cycle is an abbreviated one. In this species the fertilized ovum gives rise to a 2-cell stage of the miracidium-mother sporocyst, which possesses a propagatory cell from which 1 generation of a number of germinal cells is derived. Each germinal cell, dividing by mitosis, gives rise directly to a cercarial embryo in which a genital primordium is segregated early in development. Thus the concept of germinal lineage with polyembryony can be applied to explain reproduction in the species.

SUMMARY

The development of the miracidium, and the tracing of the germ cell cycle from the 2-cell stage of the miracidium to the genital primordium of the cercaria, was studied in *Heronimus chelydrae*. The 2-cell stage of the miracidium, which forms from cleavage of the fertilized ovum, consists of an ectodermal cell and a propaga-

tory cell. The former gives rise to most somatic tissues, while the propagatory cell gives rise to some somatic tissue and 1 generation of germinal cells within the miracidium-mother sporocyst. Germinal cells divide by mitosis and give rise to cercarial embryos with genital primordia. No meiotic divisions or fertilization stages were observed in germinal cells. The theory of germinal lineage with polyembryony is applicable in explaining reproduction in this species.

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Explanation of Figures

Abbreviations: A.Gl, apical gland; C.Gl, cephalic gland; EM, embryo; EO, eosinophil; EP, epidermal cell; ET, excretory tubule; FC, flame cell; GCM, germinal cell mass; GP, genital primodium; IC, investing cell; M, mucus; N. nerve; OC, oxyphilic cell; OS, oral sucker; P, parenchyma; PH, pharynx; PC, propagatory cell; PS, posterior sucker; SEP, subepithelium; SH, shell; VMC, vitelline membrane cell.

Figure 1. Two-cell stage of a miracidium showing an ectodermal cell in metaphase.
Figure 2. Three-cell stage of a miracidium.
Figure 3. Ectodermal cell derivatives with mitotic figures in a three-cell stage of a miracidium.
Figure 4. Five-cell stage of a miracidium.
Figure 5. Seven-cell stage of a miracidium.
Figure 6. Ten-cell stage of a miracidium with a dividing propagatory cell.
Figure 7. Eighteen-cell stage of a miracidium.
Figure 8. Section from a 23-cell stage of a miracidium showing formation of vitelline membrane cells.
Figure 9. Section near shell showing a vitelline membrane cell nucleus.
Figure 10. Section from a 46-cell embryo with epidermal cell primordia and propagatory cell derivatives.
Figure 11. Sagittal section of an embryo with approximately 125 cells with a germinal cell mass primordium.
Figure 12. Section of a 70-micron miracidial embryo.
Figure 13. Section of a 90-micron miracidial embryo.
Figure 14. Section of a 120-micron miracidial embryo.
Figure 15. Section of a germinal mass primordium from the body cavity of a 140-micron miracidial group.
Figure 16. Section of cells at the end of a germinal mass from a 150-micron embryo showing protoplasmic processes of parenchymal cells surrounding germinal cells and a 2-cell stage of a cercarial embryo.
Figures 17–19. Stages in the division of germinal cells.
Figure 20. Two-cell stage of a cercarial embryo showing a dividing micromere.
Figure 21. Section of a 6-cell stage of a cercarial embryo.
Figure 22. Section of an 8-cell stage of a cercarial embryo.
Figure 23. Section of a 16-cell stage of a cercarial embryo.
Figure 24. Section of a cercarial embryo with investing cells.
Figures 25–28. Sections of developing cercariae showing genital primordia.
Figure 29. Diagram of a fully developed cercaria showing location of the genital primordium.
Figure 30. Diagram of a 4-day-old sporocyst showing germinal cells and cercarial embryos.
Figure 31. Diagram of a 12-day-old sporocyst with developing cercarial embryos.

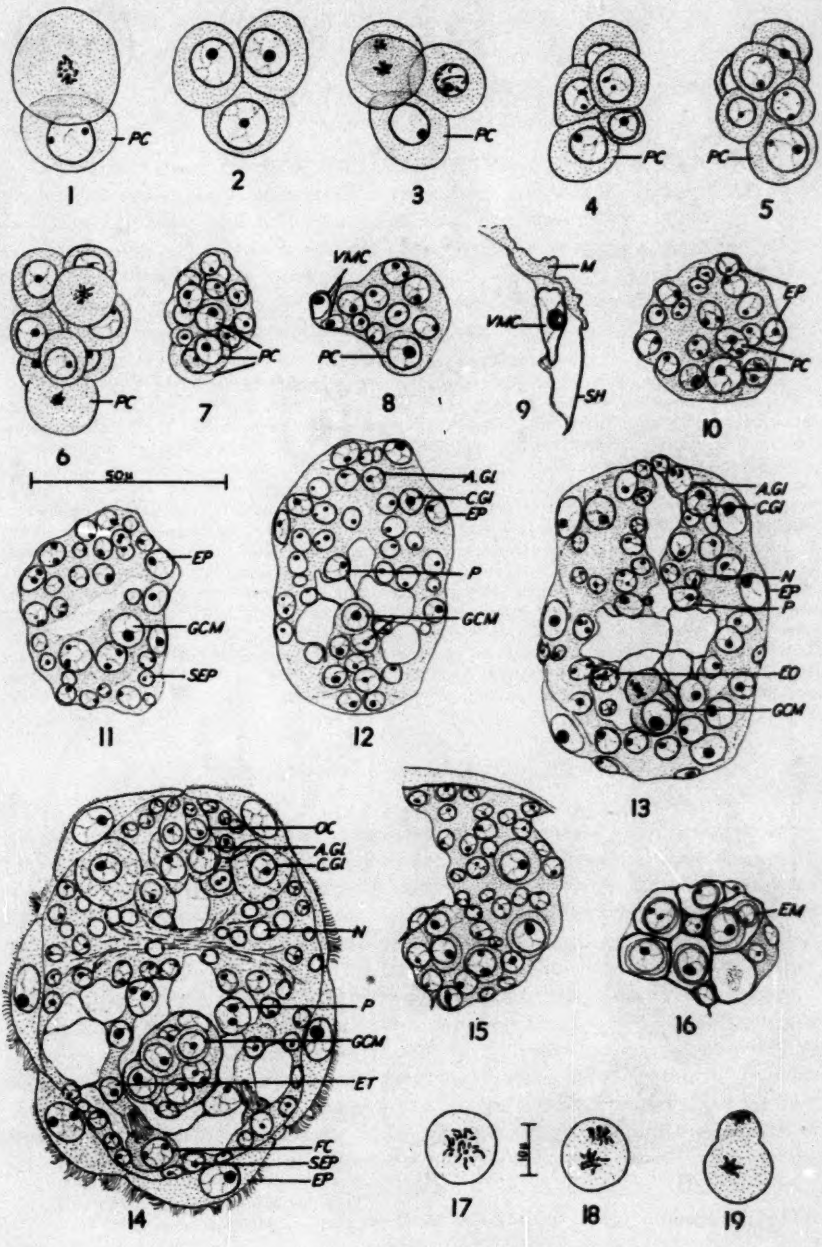
RESEARCH NOTE

LAPHYSTIUS STURIONIS PARASITIC ON COD IN NORTH AMERICAN WATERS.

On April 1, 1957, during a cruise of the Research Vessel *Albatross III* on the Northeast Peak of Georges Bank (41° 53′ N. Lat., 66° 14′ W. Long.) a cod was caught in about 45 fathoms. The fish had a sore on the proximal dorsal side of its left pectoral fin, about 4 cm in circumference and 1 or 2 mm deep. Perhaps a dozen small amphipods, roughly 2 to 4 mm in length, were moving about on the sore, apparently feeding. The cod appeared otherwise healthy and was tagged and released.

Three of the amphipods were preserved and later identified as *Laphystius* (=*Lafystius*) *sturionis* Krõyer. This species has been reported as parasitic on other fishes in New England waters (Rathbun, 1905, Occas. Papers Bost. Soc. Nat. Hist. **7**: 1–117; Holmes, 1905, Bull. Bur. Fish. **24**: 457–529) and as parasitic on cod in European waters (Dollfus, 1953, Encyclopédie Biologique **43**: 1–423). R. L. Wigley, who confirmed the identification, has found a single specimen free-living on Georges Bank. This, however, is believed to be the first record of the species parasitic on cod in the Northwest Atlantic (Specimens will be sent to U.S.N.M.) —John P. Wise, *U. S. Fish and Wildlife Service, Woods Hole, Massachusetts.*

PLATE I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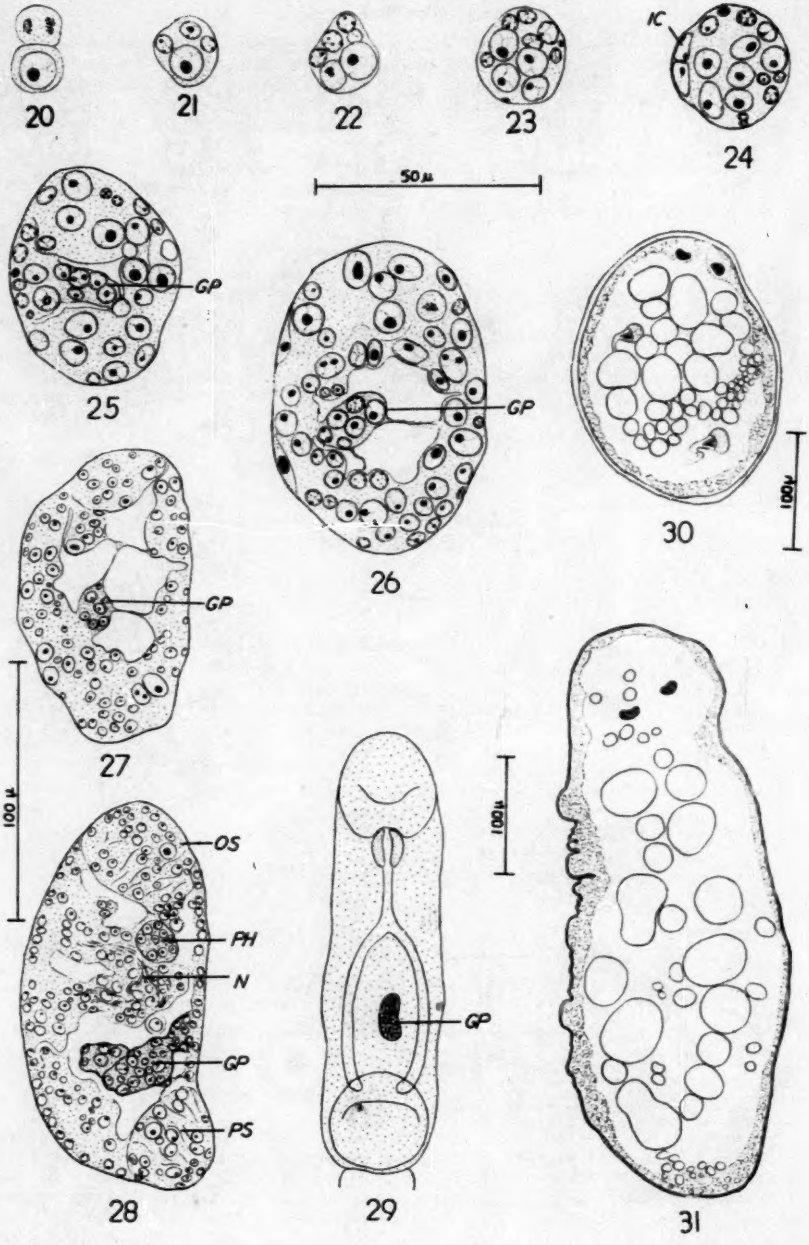
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COMPARATIVE HISTOCHEMICAL STUDIES ON CESTODES
II. THE DISTRIBUTION OF FAT SUBSTANCES IN *HYMENOLEPIS DIMINUTA* AND *RAILLIETINA CESTICILLUS**

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In an earlier paper (Hedrick and Daugherty, 1957), a report of the distribution of glycogen in the adult tapeworms, *Hymenolepis diminuta* Rudolphi, 1819, and *Raillietina cesticillus* Molin, 1858, was made. Since observations on the fat content in cestodes and certain trematodes have led some workers to assume that a certain amount of fat is a metabolic end-product of carbohydrate metabolism, the author was prompted to study histochemically the distribution and importance of lipid material in these cestodes.

MATERIALS AND METHODS

Hymenolepis diminuta were raised in the laboratory in adult male Sprague-Dawley white rats given 2 cysticercoids each 2 to 3 months previous to their sacrifice for experiments. The cysticercoids were raised in adult *Tenebrio molitor* bred in the laboratory. The rats were maintained on a diet of "Purina Laboratory Chow" and weighed between 300 and 500 grams at sacrifice. *Raillietina cesticillus* were raised in White Rock chickens of mixed sexes, 22 days of age at infection, maintained on a diet of standard commercial growing mash. Each chicken was given 10 cysticercoids which had been reared in laboratory-bred *Tribolium* sp. The number of worms recovered after 19 to 37 days of infection varied from 2 to 9. Only normal-appearing, mature worms which showed evidence of apolysis were used in this study.

For non-specific demonstration of lipid material, the worms were fixed in formol-calcium, and 1-cm lengths were embedded in gelatin, sectioned at 10 microns on the freezing-microtome, and stained with Sudan Black B. Control sections were extracted in acetone at room temperature. For demonstration of phospholipids, the acid-hematin method (Baker, 1946) was used. Controls were extracted by incubation in pyridine (as described by Baker). As an additional control, the adrenal glands of white rats were carried through the procedure along with the tapeworm tissues. These gave staining-reactions identical to those obtained by others in this laboratory.

RESULTS

The Distribution of Sudan-positive Material

Hymenolepis diminuta

The region of strobilization preceding the development of the reproductive systems contains only a small amount of fat in *Hymenolepis diminuta*, this being distributed around the osmoregulatory canals and between them in the center of the medullary parenchyma. The number and size of fat globules increases in the region containing the developing reproductive systems and in the mature region (Plate I, Fig. 1), the newly appearing fat being generally confined within the outer ring of parenchymal musculature. There are heavier deposits in the center of the medullary parenchyma adjacent to the reproductive organs and the osmoregulatory canals. There are no deposits in any of the reproductive organs or in the walls of the osmoregulatory system, although deposits occur in the interlobular parenchyma of the vitellarium and ovary. The parenchymal muscles are fat-free. In more nearly gravid proglottids, fat droplets also occur between the bundles of parenchymal muscle and in the adjacent region of the cortical parenchyma. In the

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terminal proglottids, a considerable amount of Sudan-positive material occurs in the cortical parenchyma, but the subcuticular tissue contains none. Also heavy concentrations are present adjacent to the gravid uterus (Plate I, Fig. 2). In the eggs (Plate I, Fig. 3) fat droplets are located within or just beneath the limiting membrane of the embryo and a diffusely positive reaction is given by the embryo itself. Also, finely granular lipid material is present in the membrane just beneath the egg shell. It could not be determined with certainty whether the fat droplets are located intra- or extracellularly, but it appears that some of the fat is contained within the cells. All positive material was extracted with acetone.

Raillietina cesticillus

In the immature region just behind the scolex as well as posteriorly in the region of strobilization, no fat could be demonstrated. As the reproductive systems begin to develop, however, fat is accumulated as small globules in a zone just peripheral to the parenchymal muscles. In immature proglottids this deposition occurs only in 2 parallel bands, one in the dorsal cortical parenchyma and another in the ventral cortical parenchyma. Very little is present lateral to the osmoregulatory canals. A few globules occur in the medullary parenchyma, but the developing reproductive organs as well as the walls of the osmoregulatory system are fat-free. In mature proglottids a greater amount of deposition had occurred, connecting the 2 bands of lipid in the lateral areas of the proglottid (Plate II, Fig. 1). Also more fat is present in the medullary region, but only in the parenchyma. As the proglottids become more nearly gravid, fat deposition increases, primarily in the cortical ring of fat concentration. In the gravid region there appears to be less medullary lipid, the medullary parenchyma having been largely replaced by the parenchymatous pouches containing the eggs (Plate II, Fig. 2). Very little fat is present between the cortical ring of fat and the parenchymatous pouches, and almost none is present in the subcuticular region, although in the gravid proglottids the cortical ring of lipid concentration extends to the base of the subcuticular cells. The eggs were difficult to section and few remained on most of the preparations. Of those that remained, some gave a diffuse positive reaction; others did not. It appears that some of the parenchymal fat storage is intracellular, although this was difficult to ascertain. As in *Hymenolepis,* all positive material was extracted in acetone.

The Distribution of Phospholipids

Hymenolepis diminuta

In *Hymenolepis diminuta,* dark-blue reactions indicating phospholipid occur in the subcuticular layer as numerous elongate oval or slightly irregular bodies with a small amount of diffuse positive material between them (Plate III, Fig. 1). In both cortical and medullary parenchyma, similarly shaped masses of positive substances are present but here they are not as concentrated as in the subcuticular layer and are lighter blue in color. The walls of the osmoregulatory canals, as well as those of the seminal vesicles (both external and internal), ejaculatory duct, vagina, and seminal receptacle also appear to contain phospholipid, although these do not show in the figure. The sperm and parenchymal muscles appear not to contain significant amounts of phospholipid. However, conspicuous deposits are present in the testes; this is possibly correlated with the high metabolic activity associated with sperm production. In the gravid proglottids (Plate III, Fig. 2),

some of the embryos occasionally stain intensely dark-blue, but most of them do not. Light-blue globules occur at the approximate location of the Sudan-positive material. The membrane beneath the egg shell also stains light-blue in color, but there is no indication of the granular lipid deposits present in the Sudan-stained membranes (Plate III, Fig. 3). The subcuticular and parenchymal pattern does not change throughout the length of the worm as can be seen in Figures 1 and 2 of Plate III. Pyridine-extracted material showed no positive reactions.

Raillietina cesticillus

In *Raillietina,* the distribution of phospholipid is similar. Very heavy concentrations staining dark-blue occur in the subcuticular cells, but not in the subcuticular muscles (Plate IV, Figs. 1 and 2). Also there are scattered deposits in both the cortical and medullary parenchyma. In the gravid proglottids, the parenchymal concentrations are especially evident and these stain intensely as dark-blue blotches in the medullary region adjacent to the egg-bearing pouches, as seen in Figure 3 of Plate IV. The walls of the osmoregulatory canals also give a positive reaction, but the walls of both the male and female sperm tracts, as well as the ovary, vitellarium, and sperm, apparently do not contain phospholipid. Scattered cells in the testes appear to contain phospholipid, since they stain dark-blue. No phospholipid could be demonstrated in the muscles. Because the eggs were difficult to section, no observations were made on their phospholipid content. Throughout the length of the worms, but especially in the mature and gravid regions, *Raillietina* appeared to contain more phospholipid than *Hymenolepis.* A comparison of Figures 1 and 2 of Plate III with Figures 2 and 3 of Plate IV demonstrates this clearly.

DISCUSSION

The distribution of lipids in cestodes has been studied by Brault and Loeper (1904a, b), Schiefferdecker (1874), Pintner (1922), Arndt (1922), Coutelen (1931), von Brand (1933), and Smyth (1947, 1949), using various fat-soluble dyes and the less specific osmic acid method. Of these studies, the most extensive were made by von Brand on *Moniezia expansa, M. denticulata, Taenia saginata, T. marginata,* and *Dipylidium caninum.* According to a review by von Brand (1952), the cuticle, subcuticle, and muscle fibers contain no lipid material, while the parenchyma contains considerable amounts in the form of small droplets. Only traces of lipid occur in the suckers, reproductive systems, embryos, and nerve cords. In the eggs, the lipid droplets are generally located between the embyro and the shell.

The present finding of a concentration of Sudan-positive material in the parenchymal region of *Hymenolepis diminuta* and *Raillietina cesticillus* while the organs and organ systems remain relatively free is in general agreement with the findings of the earlier workers. Since the phospholipids demonstrated with the acid-hematin methods did not, for the most part, correspond to the distribution of Sudan-positive material, it is probable that the Sudan-positive droplets observed in the present work consist mostly of neutral fats, and perhaps cholesterol. No attempt has been made to distinguish between these.

Although generally similar, an outstanding difference exists in the parenchymal distribution of Sudan-positive material in the 2 worms studied. *Hymenolepis* contains most of this material in the medullary parenchyma, while *Raillietina* shows a ring of fat concentration peripheral to the parenchymal muscles. The figures of

Smyth (1947, 1949) show a comparable variation in placement of parenchymal lipid in the 2 cultivated larval tapeworms that he studied. Plerocercoids of *Ligula intestinalis* showed a distinct peripheral distribution of the lipid droplets, even more than that demonstrated by *Raillietina*, whereas the unidentified plerocercoids of another diphyllobothriid were quite similar to *Hymenolepis*. In spite of these variations in detail, it thus becomes apparent from the present results and those of previous authors that cestodes demonstrate a basic similarity in the distribution of fatty materials: a concentration in the parenchymal regions while the more physiologically active regions are relatively free, although frequently surrounded by the lipid.

This summation suggests that a certain amount of the fat in these tapeworms can probably be regarded as an end-product of carbohydrate metabolism, as suggested by Smyth (1949) and further discussed by von Brand (1952). Smyth (1947, 1949) found that the plerocercoids of *Ligula intestinalis* and another diphyllobothriid cestode, after 7–9 days *in vitro*, produced large amounts of fat, although the fresh, uncultured larvae were nearly fat-free. In this regard, he stressed the frequent detrimental effects of the inclusion of glucose in culture media of cestodes. This effect, which has been noted in this laboratory, is in all likelihood brought on through the lowering of the pH as the result of the production of large quantities of organic acids which the worms cannot utilize completely. It may be presumed that this large amount of organic acid forces the production of longer-chained fatty acids (and thus neutral fats) through ordinary metabolic channels. Reid (1942) observed that the fat content of *Raillietina cesticillus* was the same in worms taken from fed and from starved hosts, although the tapeworms from the latter had lost most of their glycogen. From this it may be inferred that the fat stores in this worm are not available for energy metabolism. Coutelen (1931) and von Brand (1933) give further evidence of this concept through their observations of lipid globules in the excretory systems of *Echinococcus granulosus* and *Moniezia expansa*, respectively. In addition, von Brand (1933) found large droplets of fat in the uterus of *Dipylidium caninum*.

Biochemical evidence for the role of lipid in cestode metabolism is also available. Read (1952) could attribute only a portion of the carbohydrate used by *H. diminuta in vitro* to acetic acid production either under aerobic or anaerobic conditions. This observation is in accord with the idea that neutral fats are the end-products of metabolism in cestodes, since acetic acid in such a fermentative system would be only an intermediate step in the synthesis of the higher fatty acids from which neutral fats may be synthesized. Acetic acid, itself, could not be expected to be accumulated in large amounts.

No references to the histochemical demonstration of phospholipid in cestodes could be found, and the only reference to its distribution in helminths is Bullock's (1949) work on several species of *Acanthocephala*. In the present study, the major region of phospholipid accumulation in *Hymenolepis* and *Raillietina* was the subcuticular cells. Although little is known of the function of phospholipids, even in well studied forms, the possibility may be suggested, as has been done in the case of fat absorption in the mammalian intestine (Schmidt-Nielson, 1946), that this subcuticular phospholipid may be functional in fat translocation. In this regard, it is pertinent to note that a small amount of phospholipid may also be observed in the walls of the osmoregulatory system, although none has yet been

found in the lumen of this system in either *Raillietina* or *Hymenolepis*. In addition to the possible role of cestode phospholipid in fat transport, Wardle and McLeod (1952) have suggested that this substance together with cholesterol may function to promote a water balance favorable to the tapeworm by establishing colloidal conditions conducive to water uptake.

The major difference in the distribution of phospholipid between *Hymenolepis* and *Raillietina* was seen in the medullary parenchyma of the gravid proglottids, where the distribution of the substance in dark-blue staining blotches in *Raillietina* is much more marked than in a comparable area of *Hymenolepis*. Also, the subcuticular concentration of phospholipid appears to be greater in *Raillietina* throughout the length of the worm. No explanation for this difference is available. However, it may be noted that *Raillietina* was also found to be far more active in the absorption of S-35 labeled L-methionine than was *Hymenolepis* (Daugherty *et al.* in press). Whether the same may be true for fat translocation is yet to be determined.

SUMMARY

Staining frozen sections of cestodes with Sudan Black B demonstrated a constant increase in neutral fats from immature to gravid regions, the former containing little or none at all. In *Hymenolepis* the distribution of the lipid material is primarily within the medullary parenchyma, while in *Raillietina*, the greatest concentrations occur in a ring peripheral to the parenchymal musculature. The general pattern of distribution is in agreement with that described by earlier workers on other cestodes.

The distribution of phospholipid in the immature and mature regions of both worms is very similar, the heaviest concentrations being in the subcuticular cells. However, in the posterior regions, *Raillietina* contains heavy blotches of phospholipid material about the egg-bearing pouches, while *Hymenolepis* shows only slight positive reactions in this region.

ACKNOWLEDGMENTS

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EXPLANATION OF PLATES

PLATE I

FIGURE 1. A transverse section through a mature proglottid of *Hymenolepis diminuta* showing the distribution of Sudan-positive material in the medullary parenchyma. Sudan Black. 20×.

FIGURE 2. A transverse section through the gravid region of *Hymenolepis diminuta* showing the heavy concentrations of Sudan-positive material adjacent to the uterus. Sudan Black. 100×.

FIGURE 3. Sectioned eggs of *Hymenolepis diminuta* showing lipid deposits either within the embryo or adjacent to it, and finely granular lipid material in the membrane just beneath the egg shell. Sudan Black. 450×.

PLATE II

FIGURE 1. A transverse section through a mature proglottid of *Raillietina cesticillus* showing a band of Sudan-positive material in the cortical parenchyma. Sudan Black. 100×.

FIGURE 2. A transverse section through the gravid region of *Raillietina cesticillus* showing the placement of Sudan-positive material in the cortical parenchyma. Sudan Black. 60×.

PLATE III

FIGURE 1. A transverse section through the mature region of *Hymenolepis diminuta* showing globular deposits of phospholipid in the subcuticular cells, testes, and the walls of the osmoregulatory canals and scattered deposits in the parenchyma. Acid-hematin method. 80×.

FIGURE 2. A transverse section of the gravid region of *Hymenolepis diminuta* showing phospholipid deposits (black) in the subcuticular layer and adjacent to the uterus. Sudan-positive material appears on the photograph as grey deposits. Acid-hematin method. 100×.

FIGURE 3. Sectioned eggs of *Hymenolepis diminuta* showing the staining reaction (light grey on photograph) of the membrane beneath the eggshell. Acid-hematin method. 480×.

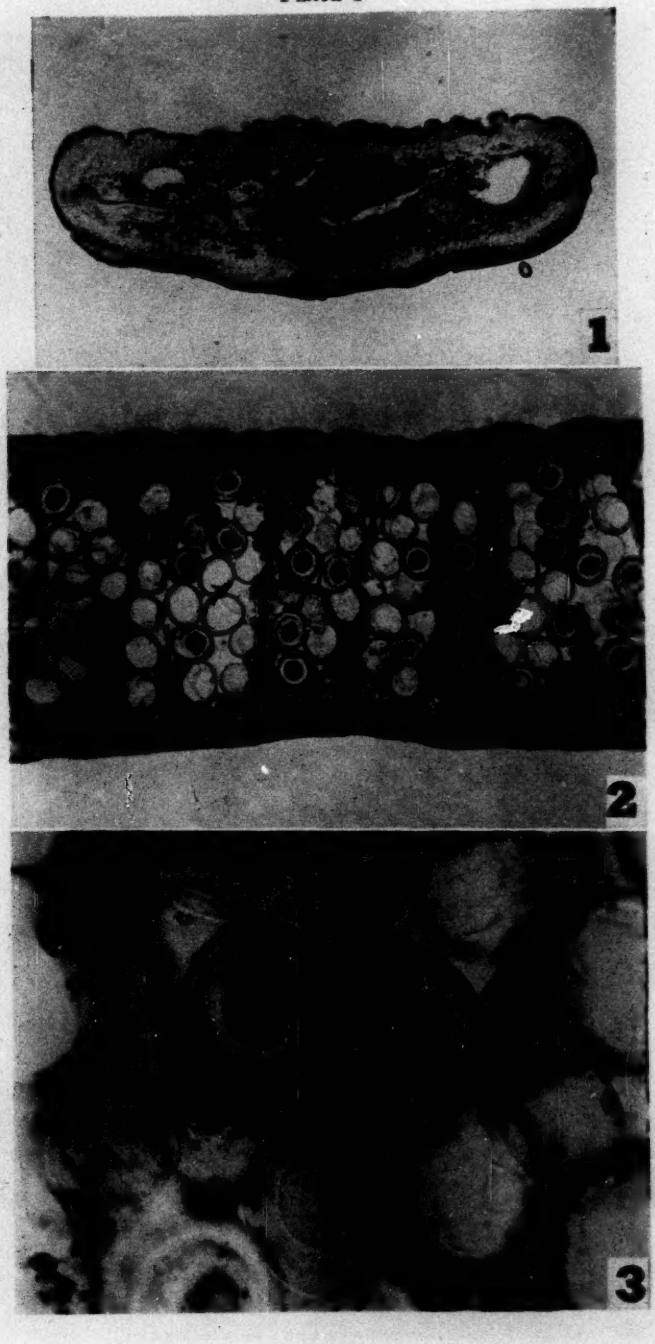
PLATE IV

FIGURE 1. Transverse section through the immature region of *Raillietina cesticillus* showing the heavy subcuticular concentrations of phospholipid. Acid-hematin method. 100×.

FIGURE 2. Transverse section of a mature proglottid of *Raillietina cesticillus* showing phospholipid concentrations in the subcuticular cells, testes, and the walls of the osmoregulatory canals. Acid-hematin method. 100×.

FIGURE 3. Transverse section through the gravid region of *Raillietina cesticillus* showing heavy subcuticular and medullary concentrations of phospholipid. Acid-hematin method. 80×.

PLATE I



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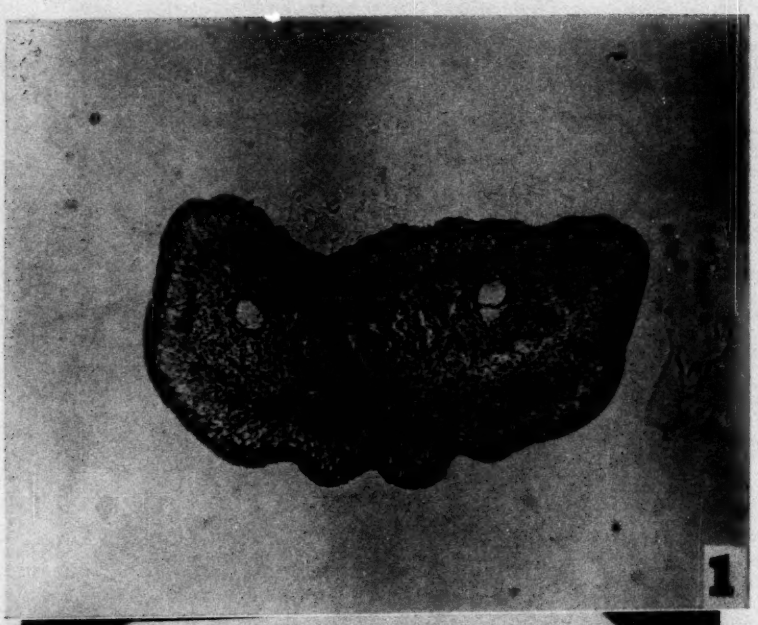

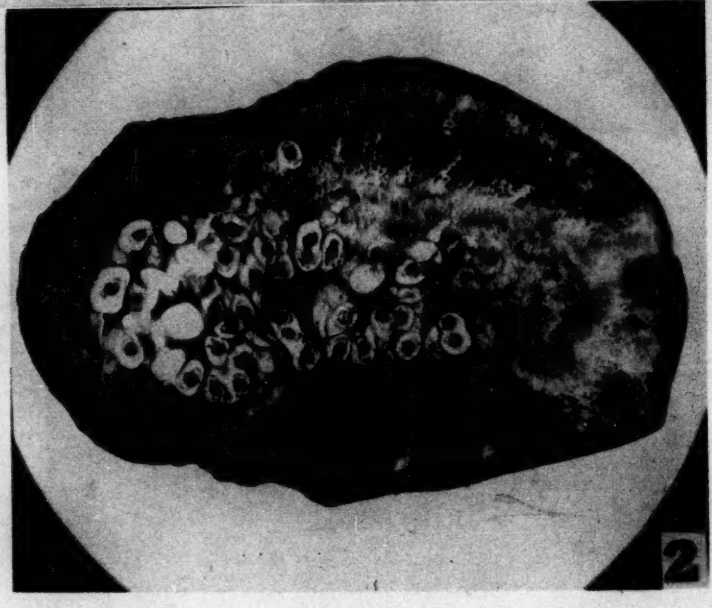

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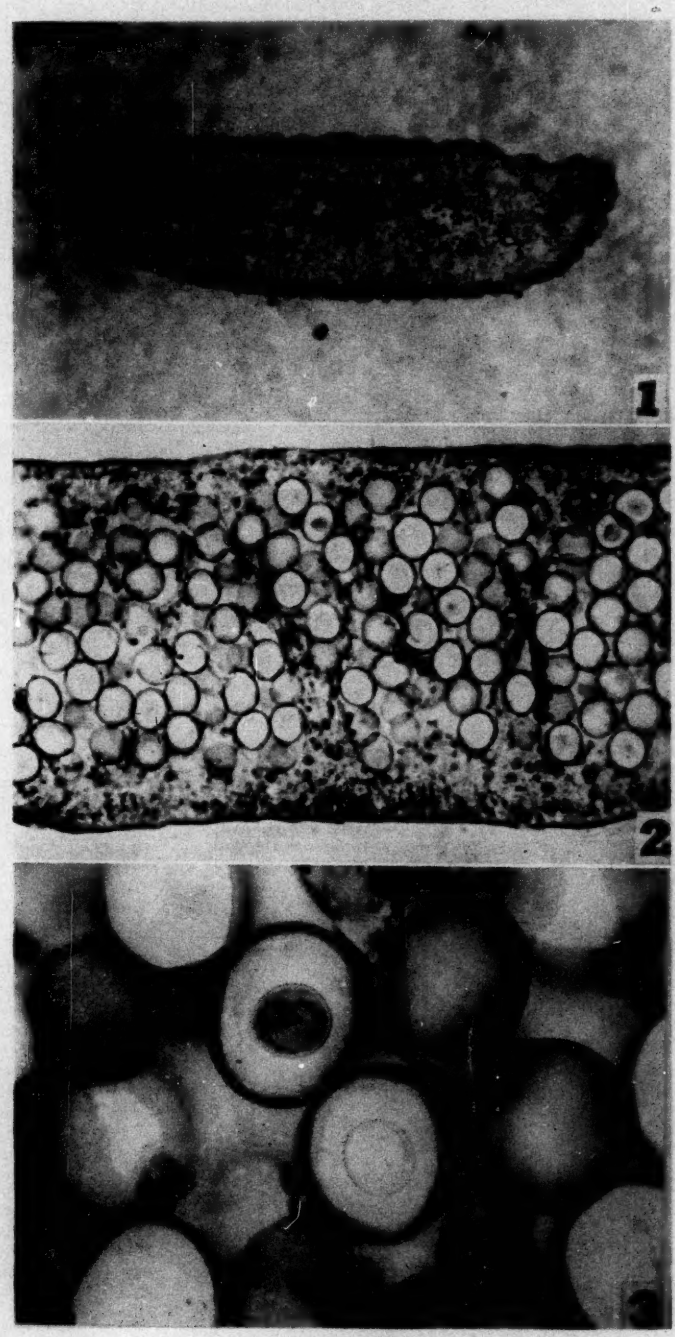
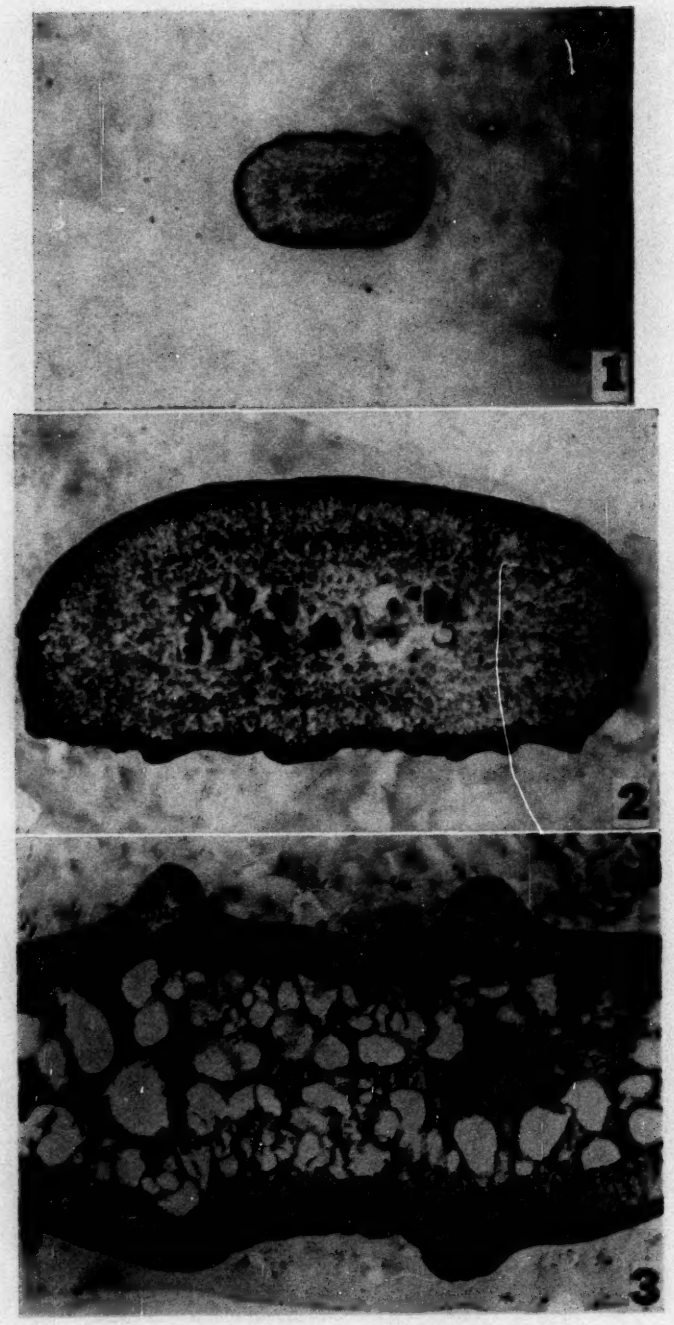


PLATE IV



EFFECT OF COBALT-60 AND X-RAY ON INFECTIVITY OF *ASCARIS* EGGS*

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We have been interested in the feasibility of using ionizing radiations as a possible means of reducing or controlling certain parasitic infections of man and animals, particularly those transmitted by contact with contaminated soil or by ingestion of contaminated food.

Investigators in food technology have been exploring the possible use of radiation in the sterilization of food by the application of doses of radiation sufficient to inhibit the growth of microorganisms, such as bacteria, molds, and yeasts. Information on the effect of similar radiations on food-borne parasites is therefore desirable.

Stoll (1947) estimated that over 600 million people are infected by *Ascaris lumbricoides*. In Japan, according to Pan and associates (1954), application of night soil in the late fall, winter, and spring results in an accumulation of embryonated eggs of *Ascaris* in the soil and on the vegetables by late spring. Yamaguchi (1925) and Sugita (1926) found that up to 80% of the vegetables sampled were contaminated with viable *Ascaris* eggs. The vegetables sampled were from the first crops of the year and included cabbage, spinach, green onions, radishes, and turnips. In Japan these vegetables are mainly consumed in the raw or pickled state. Fueki (1952) examining 2,250 samples of 20 kinds of vegetables, made similar observations.

The present study was designed to determine the effect of cobalt-60 and x-radiation on the infectivity of *Ascaris* eggs.

MATERIALS AND METHODS

The radiologic techniques applied were similar to those employed in our work with *Trichinella spiralis*, in which the life cycle was broken by irradiation of the larvae with x-ray or cobalt-60 (Gould *et al*, Gomberg and Gould, 1953).

Adult forms of *Ascaris lumbricoides suum* were obtained from a local abattoir. Large females were selected, washed several times in normal saline solution to remove adherent mucus and detritus, and placed in the refrigrator at 7° C overnight. The worms were then placed in a pan containing normal saline solution. With a pair of fine scissors the body wall was punctured dorsally at a point above the vulva and a longitudinal incision was made from the point of puncture to the posterior end.

The uterus was cut into 3 portions and eggs from each portion were examined for their appearance and evidence of fertility. The distal or terminal portion of the uterus contained relatively few infertile eggs compared with the more proximal portions. Since eggs removed from the uterus tended to clump, and to adhere to the dissecting needle and the glass container, the external albuminoid coating (Fig. 1) of the eggs was removed (Fig. 2) by placing them in a solution of 10% NaOCl (Antiformin) for 15 to 20 minutes. The eggs were next washed

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with several changes of distilled water to remove the antiformin and debris, and then washed daily for 1 week with distilled water, until used. When the eggs were not being treated or washed, they were kept in the ice box at 7° C.

Guinea pigs weighing about 400 grams (and in preliminary experiments, white rats weighing about 200 grams) were tube-fed with counted numbers of infective *Ascaris* eggs incubated 25 days at 30° C) exposed to various doses of cobalt-60 at a dose rate of 623 3 roentgens equivalent physical (rep) per minute. (Alicata, 1934, observed that *Ascaris* eggs were infective after the embryo had undergone a molt.) The animals were killed after approximately 8 days, by exposure to ether. The gross appearance of the trachea, lungs, and liver was noted. The trachea was examined under the dissecting microscope for presence and number of larvae. Sections were taken from the liver and lung for microscopic study for the presence of larvae and for evidence of inflammatory reaction. The rest of the lung tissue was triturated in saline solution in a Waring Blendor for 30 seconds. The suspension was then examined fractionally for presence and number of *Ascaris* larvae.

EXPERIMENTS AND RESULTS

Experiment 1.

Effect of radiation of eggs on development of motile embryos. Unsegmented *Ascaris* eggs suspended in distilled water were placed in lucite vials and irradiated with 3,000, 10,000 and 100,000 rep cobalt-60. They were then placed in glass containers and incubated at 30° C. Non-irradiated eggs from the same stock were incubated at the same temperature (Figs. 3, 4). Direct observations were made on *Ascaris* eggs for evidence of morphologic changes at various periods of incubation following irradiation. With 100,000 rep, only about 1% of embryos developed (Fig. 5) even after 21 days' incubation.

Experiment 2.

Test of relative susceptibility of white rat and guinea pig to larval Ascaris *infection.* Graham (1937) found that the guinea pig was more susceptible than the rat to pulmonary infection with *Ascaris* larvae. Inasmuch as it would have been more convenient to use the rat as the experimental host, we decided to test the relative susceptibility of the two animals. Accordingly, 16 white rats and 8 guinea pigs were each infected with 5,000 to 50,000 infective *Ascaris* eggs and sacrificed after 6 to 8 days. Two non-infected rats served as controls. The liver and lungs were examined grossly, and tissue was taken from each of these organs for microscopic section. The remaining lung tissue was triturated in normal saline solution in a Waring Blendor for recovery of larvae.

None of the rats died. Seven of 12 rats fed 5,000 to 20,000 eggs actually gained (from 10 to 32 g) in weight when sacrificed 6 to 8 days after infection; the other 5 lost weight (from 4 to 40 g). Three of 4 rats fed 30,000 or 50,000 eggs lost weight (12 to 24 g); the fourth rat showed no change in weight. In only 2 of the 16 rats were any larvae recovered from the trachea (in one, 3; in another, 2 larvae). The numbers of eggs fed and the average numbers of larvae recovered from the lungs of the rats were as follows: 5,000 eggs, 18 larvae; 10,000 eggs, 44; 20,000 eggs, 6; 30,000 eggs, 16; and 50,000 eggs, 10 larvae.

Five of the 8 guinea pigs died 6 to 8 days after infection. Seven showed loss of weight (12 to 128 g, average 72 g). The guinea pigs that were fed 50,000 eggs both died 6 days after infection; their lungs were grossly hemorrhagic. The numbers of eggs fed and the average numbers of larvae recovered from the lungs of the guinea pigs were as follows: 5,000 eggs, 273 larvae; 10,000 eggs, 1,180; 20,000 eggs, 4,578; 30,000 eggs, 7,050; and 50,000 eggs, 12,400 larvae.

These comparative findings in rats and guinea pigs, therefore, amply confirm the work of Graham (1937).

Microscopic sections of liver in both species of animals showed only few focal granulomatous areas. Sections of lung showed relatively mild lesions in rats (Fig. 6), and moderately severe or severe pneumonitis with various degrees of hemorrhage in guinea pigs (Figs. 7–9).

In view of the far greater susceptibility of the guinea pig than the rat to larval pulmonary ascariasis, it was decided to utilize the guinea pig as the experimental animal in subsequent experiments.

Experiment 3.

Effect of various doses of cobalt-60 on infectivity of Ascaris *eggs.* Infective *Ascaris* eggs were exposed to various doses of cobalt-60 and then fed to guinea pigs, in order to determine the dose of radiation necessary to prevent larval infection in lungs of guinea pigs. Each guinea pig was fed about 20,000 infective *Ascaris* eggs. Four groups of 3 to 5 guinea pigs were infected with eggs exposed to doses of cobalt-60 ranging from 3,000 to 500,000 rep (see Table I). In each group, 3 control

TABLE I. *Effect of exposure of infective* Ascaris *eggs ("denuded" unsegmented eggs incubated 25 days at 30° C; embryo within eggs observed to have molted) to various doses of cobalt-60 on larval infection of lungs of guinea pigs.*

Dose of cobalt-60 rep	No. of guinea pigs*	Average number of larvae recovered from		Degree of hemorrhage and/or inflammation in lungs at autopsy
		Trachea	Lungs	
0	3	49	4,348	++++
3,000	4	81	10,128	++++
10,000	3	29	5,417	++++
0	3	311	6,340	++++
30,000	5	6	2,755	++++
100,000	4	1	0	++
0	3	80	1,313	++++
100,000	5	1	6	++
150,000	4	0	0	+
0	3	165	4,183	++++
250,000	5	1	0	+
500,000	5	0	0	0

* Each animal was fed about 20,000 infective *Ascaris* eggs and was killed 8 days after infection or died 5 to 8 days after infection.

animals were fed the same number of non-irradiated eggs. The procedures were the same as in Experiment 2.

Feeding of eggs exposed to cobalt-60 in doses ranging from 3,000 to 30,000 rep showed no difference in effects produced compared to those from non-irradiated eggs. All these test and control animals showed loss of weight, severe respiratory distress, and a number died between 5 and 8 days after infection. In general, the number of larvae recovered from the lungs of guinea pigs fed with eggs exposed to 30,000 rep was comparable to the number recovered from control animals (Table I).

A dose of 100,000 rep cobalt-60, however, was effective in reducing the number of larvae recoverable from the lungs to only a few, compared with several thousand larvae recovered in controls. Nine guinea pigs that were fed eggs exposed to 100,000 rep, showed little or no loss in weight 8 days following infection, but numerous petechiae were seen grossly beneath the pleural surfaces of the lungs. Microscopically, the sections of lungs had focal granulomatous pneumonitis producing consolidation of less than half the total area of parenchyma. Most sections

were free of hemorrhage or of recognizable larvae, although often there was some foreign-body reaction, apparently about larvae that had degenerated or were being destroyed.

Guinea pigs fed eggs exposed to a dose of 250,000 rep maintained their weight, and those fed eggs exposed to 500,000 rep actually gained weight. When doses of 150,000 rep or over were applied to the eggs, no larvae were recovered from the lungs. The lungs of most animals fed eggs exposed to 500,000 rep of radiation appeared grossly normal; microscopic sections of lungs of these animals disclosed mild focal granulomatous pneumonitis affecting about 10% or less of the parenchyma.

While the feeding of 20,000 non-irradiated eggs was fatal to 8 of the 12 control guinea pigs in from 5 to 8 days after infection, none of the guinea pigs fed this number of eggs exposed to 100,000 rep or more of cobalt-60 died. A dose of 150,000 rep cobalt-60 applied to infective *Ascaris* eggs was effective in preventing larval infection in the lungs of guinea pigs.

Experiment 4.

Effect of irradiation of unsegmented Ascaris *eggs upon their infectivity.*

Unsegmented *Ascaris* eggs were exposed to cobalt-60, incubated for 3 to 4 weeks until infective and then fed to guinea pigs. Eggs from the same stock were irradiated after they became infective and were then fed to animals. At the same time non-irradiated infective eggs were fed as controls. Each animal was fed about 20,000 eggs and was sacrificed after 8 days. Blocks of tissue from the lung were taken for microscopic section and the rest of the lung was triturated and suspended in saline solution in order to make larval counts. *Ascaris* eggs in the infective stage were more sensitive than unsegmented eggs to cobalt-60 in doses of 30,000 and 100,000 rep, as judged by number of larvae recovered from the lungs of infected guinea pigs.

Experiment 5.

Effect of x-radiation on infectivity of Ascaris *eggs.* One experiment was conducted in which infective eggs were exposed to x-radiation from a 245-Kv machine (18 ma; 0.5-mm copper and 1-mm aluminum filter; dose rate, 612 r per minute). Five groups of 5 guinea pigs each were fed about 20,000 infective eggs exposed to 3,000, 10,000, 30,000, 50,000 and 100,000 r, 6 controls were each fed about 20,000 non-irradiated eggs and 2 controls were fed no eggs. The results seemed to indicate that x-radiation was more effective than cobalt-60, since fewer larvae were recovered from the lungs of animals fed eggs exposed to doses ranging from 3,000 to 50,000 r than from eggs exposed to the same doses of cobalt-60. No larvae were recovered from guinea pigs fed eggs exposed to 100,000 r.

DISCUSSION

Kahlstorf (1928) found that an increasing percentage of fertile *Ascaris* eggs was damaged by increasing doses of x-ray, but that all were not killed by doses as high as 19,800 r. Braun (1930) determined that time was an important factor in the amount of damage to *Ascaris* eggs produced by a given quantity of x-ray or gamma rays of radium. Cook (1939) reported that *Ascaris* eggs exposed to 5,000 r x-ray did not form embryos if allowed to develop at room temperature, but that

they recovered from the damage of the radiation if, following exposure, they were kept at a temperature of 0 to 5° C for ½ to 6 months. Bachofer and Pahl (1955), working with *Ascaris lumbricoides suum*, and Tahmisian, Devine and Wright (1955), working with *Ascaris megalocephala*, were unable to confirm the findings of Cook. The latter workers, however, felt that 5,000 r x-ray destroyed the capacity of the egg to embryonate.

In our experiments, in which eggs were fed to guinea pigs immediately after radiation, some of the fertile eggs of *Ascaris* were not killed even by doses as high as 150,000 and 250,000 rep cobalt-60. Some degree of pneumonitis was produced and occasionally a larva was recovered from the trachea or lung, even after exposure of eggs to these doses. In the single experiment with 245 Kv x-radiation, on the average 12 larvae were recovered from the lungs of 5 guinea pigs fed 20,000 infective eggs, compared to an average of 4,540 recovered from the lungs of 6 control animals fed non-irradiated eggs.

While the experiments have all been on atypical eggs, i.e., those from which the albuminoid coating had been removed, nevertheless our findings suggest that in irradiation of food contaminated with *Ascaris* eggs, the effective amount of cobalt-60 would be more than 150,000 rep, and of 245 Kv x-radiation over 100,000 r. Additional radiation would have to be allowed to insure penetration of the full thickness of the material to be irradiated.

SUMMARY

The effect of cobalt-60 was tested on development of unsegmented eggs of *Ascaris lumbricoides suum*, and of cobalt-60 and x-radiation on the ability of embryonated eggs to produce larval pulmonary infection in guinea pigs. After removal of their albuminoid outer coats, unsegmented *Ascaris* eggs were exposed to cobalt-60 and incubated for various periods at 30° C. Doses of 30,000, and 100,000 rep retarded development of embryos. Embryonated eggs seemed to be more sensitive than unsegmented eggs to radiation with cobalt-60.

The observation of Graham, that the guinea pig is much more susceptible than the white rat to pulmonary larval infection with embryonated *Ascaris* eggs, was confirmed.

A dose of 100,000 to 150,000 rep cobalt-60 applied to infective (embryonated) *Ascaris* eggs was necessary to prevent development of viable larvae in the lungs of the guinea pig. After a dose as high as 250,000 rep, some degree of pneumonitis was still produced following feeding of the eggs to guinea pigs.

A dose of 100,000 roentgens (r) x-ray (245 Kv.) was effective in preventing larvae from developing in the lungs of guinea pigs.

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EXPLANATION OF PLATES

FIGURES 1–4. Non-irradiated eggs of *Ascaris lumbricoides suum*. ×300.

FIGURE 1. Unsegmented eggs, showing irregular outer albuminoid coating.

FIGURE 2. Unsegmented eggs from which albuminoid material was removed by washing in 10% NaOCl.

FIGURE 3. Eggs after 8 hours' incubation at 30° C. Most are in 2- or 4-cell stage.

FIGURE 4. Eggs after 18 days' incubation. Most eggs are embryonated.

FIGURE 5. *Ascaris* eggs that had been irradiated with 100,000 rep cobalt-60, and then incubated at 30° for 17 days. A few cells are unsegmented, but most of them show abnormal segmentation.

FIGURE 6. Granulomatous pneumonitis and cross-sections of *Ascaris* larvae in lung of rat 6 days after it was fed 50,000 infective *Ascaris* eggs. ×320.

FIGURE 7. Granulomatous pneumonitis and cross-sections of *Ascaris* larvae in bronchiole of guinea pig, 8 days after animal was fed 10,000 infective *Ascaris* eggs. ×240.

FIGURE 8. Pneumonitis and section of *Ascaris* larvae in lung of guinea pig, 8 days after animal was fed 10,000 *Ascaris* eggs. ×340.

FIGURE 9. Portion of *Ascaris* larva with associated hemorrhage and pneumonitis in guinea pig, 8 days after animal was fed 20,000 infective *Ascaris* eggs. ×480.

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PLATE I

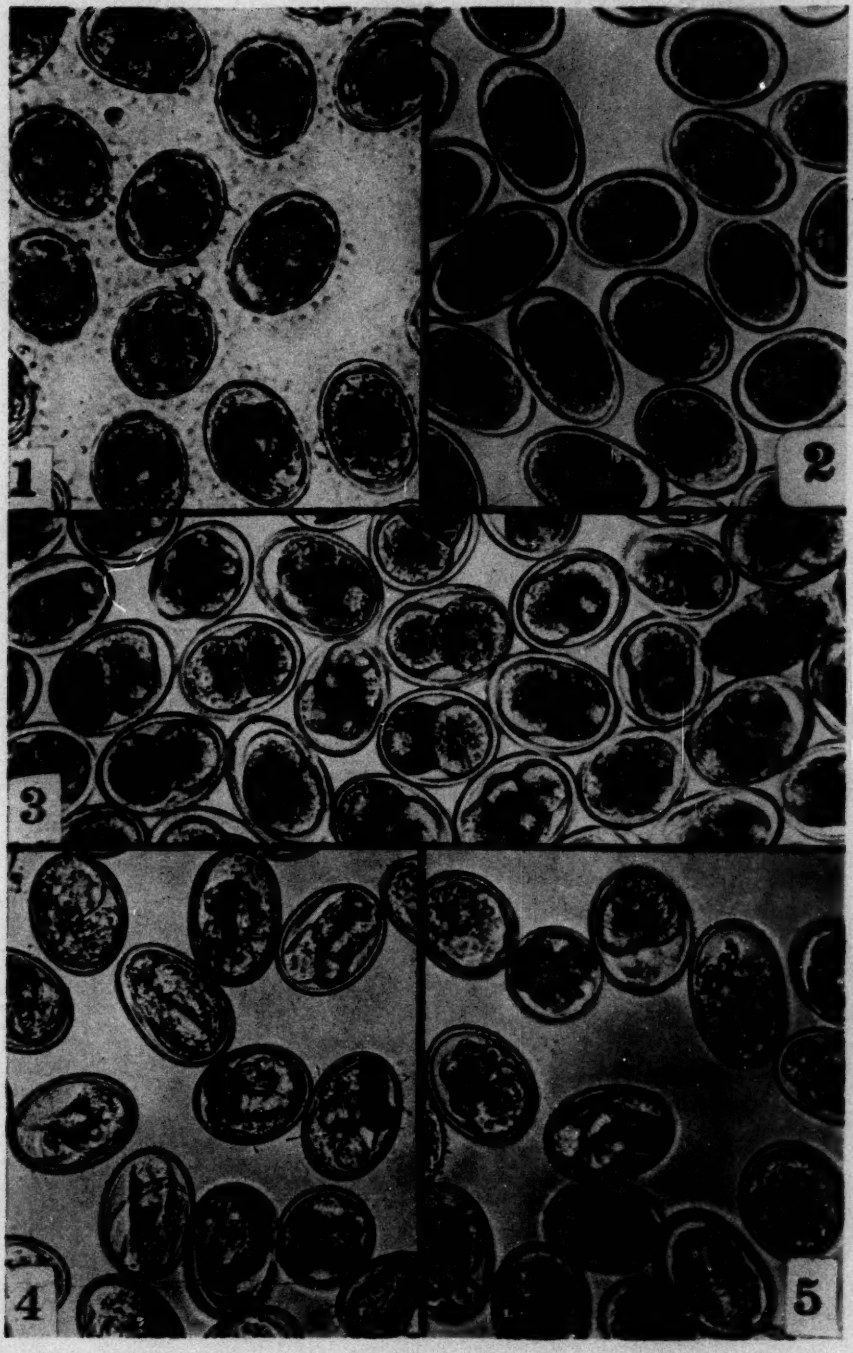
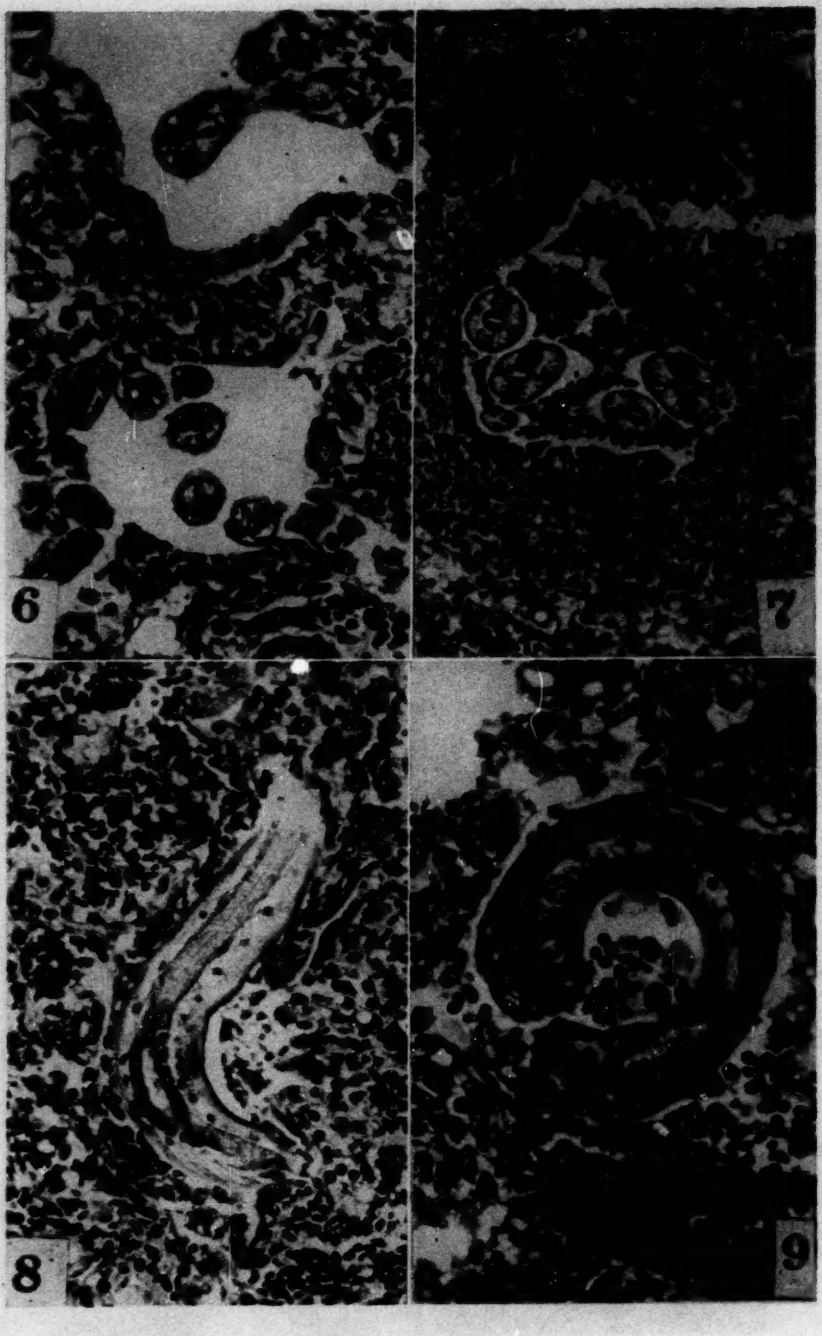


PLATE II



VARIATION IN *LOXOTHYLACUS PANOPAEI* (GISSLER), A COMMON SACCULINID PARASITE OF MUD CRABS, WITH THE DESCRIPTION OF *LOXOTHYLACUS PERARMATUS*, N. SP.

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The first species of *Sacculina* (*sensu lato*) to be reported from North American waters was *Sacculina panopaei* Gissler, found on the crab *Panopeus herbstii* H. Milne-Edwards in the vicinity of Tampa, Florida (Gissler, 1884). Nothing further was published about this rhizocephalid until Boschma (1928) gave the first account of its internal anatomy and transferred the species to a new genus, *Loxothylacus*, which differs from *Sacculina* chiefly in having the attachment of the visceral mass at some distance from the stalk. *Loxothylacus panopaei* is now known to occur on a variety of Xanthidae (Mud Crabs) in the Gulf of Mexico and the Caribbean and has also been found on the Pacific Coast at Nanaimo, British Columbia.

Although present records indicate that the parasite described by Gissler is one of the most common and widely distributed species of North American Sacculinidae, the anatomical features of only a few specimens have been noted in detail. In order to determine the extent of variability of this species, the writers studied 23 specimens from 5 distinct localities and 3 different hosts. The present paper also includes the description of *Loxothylacus perarmatus*, a new species found on the crab, *Parthenope pourtalesi* (Stimpson), from the Gulf of Mexico.

Loxothylacus panopaei (Gissler)
(Plate I)

Material examined:

Englewood, Florida, Zoological Research Supply coll. 1941, 3 specimens on *Panopeus herbstii* H. Milne-Edwards.

Bay St. Elaine, Louisiana, J. W. Hedgpeth coll. 1948, 2 specimens on *Panopeus herbstii* H. Milne-Edwards.

Bassa Bassa Bay, Louisiana, Dan Wray coll. 1948, 13 specimens on *Eurypanopeus depressus* (Smith).

Bay Chene Fleur, Louisiana, Dan Wray coll. 1948, 4 specimens on *Eurypanopeus depressus* (Smith).

Head of Copano Bay, Texas, J. W. Hedgpeth coll. 1947, 1 specimen on *Rhithropanopeus harrisii* (Gould).

External Anatomy

The parasites are reniform, convex in the region of the mantle opening and concave at the stalked surface. Usually there is a slight mid-dorsal and mid-ventral lobe. The mantle opening is always displaced somewhat to the left side, which is the side facing the thorax of the host, and is usually surrounded by a more or less elevated boundary. A short, heavily chitinized stalk, having an average diameter of 0.5 mm, attaches the parasite to the abdomen of the host.

The dimensions of the adult animal vary not only with the presence or absence of eggs in the mantle cavity but also with the kind of host. Specimens infesting *Panopeus herbstii* are proportionally larger than those found on *Eurypanopeus depressus*, which is a smaller crab. The largest specimen measured 4.5 mm in length,

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8.2 mm in thickness (dorso-ventral diameter) and 3.7 mm in breadth. The average measurements of 14 specimens from various hosts were: length 2.8 mm, thickness 4.4 mm, breadth 1.9 mm.

Minute hair-like excrescences or spines occur on the external cuticle of the mantle (Figs. 2, 3, 4). They measure 6 to 20 microns on parasites of *Rhithropanopeus harrisii* and of *Eurypanopeus depressus* but attain a length of 44 microns on the parasites of *Panopeus herbstii*. With the exception of a specimen infesting *P. herbstii* from Bay St. Elaine, Louisiana, all have spines with the tips divided into 2 or 3 points. Those of the exceptional animal have rounded tips (Fig. 4). In all cases the spines are beset with lateral hairs that project upward.

Internal Anatomy

The thickness of the mantle, including the external cuticle, varies from 48 to 120 microns, with the cuticle measuring 8 to 26 microns. Except for the well-developed sphincter around the mantle opening, the mantle musculature is comparatively weak. Its fibers interlace and appear to run in all directions. The connective tissue of the mantle consists of collagen-like fibers, united at regular intervals into bundles which serve as supporting elements. They pass through the mantle, connecting the outer with the inner cuticle. Tonofibrils formed from epidermal cells are in close proximity to these colonnades and probably strengthen them.

Numerous retinacula (Fig. 7) occur on the thin internal cuticle which lines the mantle and covers the visceral mass. They consist of a slight basal prominence from which arise 3 to 10 barbed spindles, 3 being the most frequent number. The spindles are 3 to 22 microns in length and 2 to 6 microns in diameter.

A diagnostic feature of the genus *Loxothylacus* is the attachment of the mesentery a short distance to the right of the stalk which causes the visceral mass to lie obliquely in the mantle cavity (Fig. 5). This is well exemplified in every specimen we examined.

The organs of the visceral mass are embedded in a connective tissue framework with blood lacunae present principally in the region of the stalk and mesentery. A few muscle fibers pass transversely through the mass and others form a thin layer close to the surface. The ovary, which is the most voluminous portion of the visceral mass, contains groups of oocytes enclosed within thin-walled follicles.

Two bilobed colleteric glands occupy a position in the anterior half of the visceral mass just above the mid-line. They are embedded in the surface of the mass but frequently protrude somewhat into the mantle cavity. A single colleteric gland consists of a well-developed dorsal and ventral system of branched canals whose tubes converge as they pass to the center of the gland. The tubes possess a moderately thick lining which stains differentially from the internal and external cuticle when Mallory's stain is employed. Generally, the number of tubes on the dorsal and ventral half of the gland is approximately equal. However, in 1 specimen on *Eurypanopeus depressus* the right colleteric gland has 22 tubes on the dorsal half and 52 tubes on the ventral half. In 19 specimens, the greatest number of tubes seen in a single cross-section of the right gland varies from 22 to 45 and in the left gland from 28 to 57, but the average number for either the right or left gland is the same, namely, 34.

The male genital organs lie in the posterior part of the visceral mass (Figs. 1 and 5). When both testes are fully developed, they resemble 2 retorts lying side by side. The bulb of the retort is the testis proper, a small, strongly curved sac; the tubular portion is the vas deferens. Although normally the testes are separate, in 1 specimen they are fused at their distal ends, forming a common lumen. The testicular sacs have a diameter which attains up to 700 microns in well-developed organs, with a wall that varies from 32 to 80 microns in thickness. While these organs tend to be spherical in shape, they are subject to lateral compression in many cases. Boschma (1931) reported that in *L. panopaei* one of the testes is usually larger than the other. This was confirmed by us in 12 of 18 specimens examined for this feature; the other 6 had both testes equally well developed. In 7 specimens it was the right testis that was rudimentary and in 5 the left. The main body of the testis curves dorsad then bends sharply ventrad and merges, sometimes tortuously into the much narrower vas deferens which proceeds in a ventro-lateral direction as a straight tube whose lumen is plugged with chitin.

The vasa deferentia do not open directly into the mantle cavity but into smaller cavities, which shall be referred to as "vestibules" (Fig. 6). The vestibules have their own opening into the mantle cavity. Their cuticle is the same thickness as the inner cuticle of the mantle, except in the region where the vasa deferentia enter and where the vestibules open into the mantle cavity. At these points the cuticle is much thicker and possesses spines of considerable length (11 to 20 microns).

A single ganglion is located slightly anterior to the colleteric glands and in the plane which bisects the stalk and mantle opening.

Previous Host Records and Distribution: On *Panopeus herbstii* H. Milne-Edwards. Tampa, Florida (Gissler, 1884); Jamaica, B. W. I. and Cuba (Boschma, 1928); Englewood Florida, occurring as conjoined twins (Reinhard, 1954). On *Panopeus occidentalis* Saussure. Puerto Rico (Boschma, 1928); St. Croix, Virgin Islands (Boschma, 1931). On *Eurypanopeus depressus* (Smith), Florida and Texas (Boschma, 1928); Grand Isle, Louisiana (Causey, 1954). On *Rhithropanopeus harrisii* (Gould), Grand Isle, Louisiana (Behre, 1950). On *Tetraplax quadridentata* (Rathbun), Venezuela (Boschma, 1931). On *Lophopanopeus bellus* (Stimpson), Nanaimo, British Columbia (Boschma, 1931).

New Host Record: On *Tetraxanthus rathbunae* Chace, 28° 30′ N., 85° 36′ W., Gulf of Mexico, "Oregon" Station 36, 120 fathoms. June 27, 1950, U. S. Fish and Wildlife Service coll. Two parasites on 2 hosts. U.S.N.M. Cat. No. 91149.

Loxothylacus perarmatus, n. sp.
Plate II

Holotype: One specimen on *Parthenope* (*Platylambrus*) *pourtalesi* (Stimpson). 22° 27′ N., 89° 34′ W., Gulf of Mexico, "Oregon" Station 218, 124 fathoms, Jan. 10, 1951, U. S. Fish and Wildlife Service coll. U.S.N.M. Cat. No. 93688.

Description: The parasite is reniform, with an elevated mantle opening displaced slightly to the left side (Fig. 8). The short stalk measures 2.3 mm in diameter and is heavily chitinized. The parasite's dimensions are: length 12 mm, thickness 18 mm and breadth 8 mm.

The external cuticle is a thick layer of chitin possessing many excrescences, furrows and pits containing a chitinous plug. It varies in thickness from 60 to 100 microns, but in the region of the stalk it may become 200 microns thick. The excrescences are long narrow spines on the right side of the animal, 24 to 60 microns in height and 2 to 4 microns in width at the base (Fig. 13). On the left side, facing the thorax of the host, they are 10 to 18 microns in height and 8 to 10 microns in width at the base (Fig. 12). The furrows of the cuticle are 40 to 160 microns in depth, 40 to 100 microns in width and may run parallel to each other or branch irregularly (Fig. 9). Shallow pits containing a chitinous plug occur only on the cuticle of the left side of the parasite. These peculiar structures, which may be similar to the "lime corpuscles" mentioned in descriptions of various sacculinids, consist of a small hyaline basin

into which is fitted a discoidal chitinous plug, the top of which is on a level with the surface of the cuticle (Figs. 10 and 11). In cross-sections the hyaline layer is 8 microns thick. The disc has a uniform diameter of 40 to 80 microns and a depth of 28 to 40 microns. The cuticular excrescences in this area show some modification from the ordinary type.

The retinacula which occur on the thin internal cuticle consist of 20 to 40 spindles arising from a somewhat tapering columnar base which has a maximum diameter of 26 microns and a maximum height of 24 microns (Fig. 15). The spindles are 20 to 22 microns in length and possess lateral barbs.

The mantle projects into the mantle cavity in the form of numerous folds from 160 to 480 microns in height (Fig. 14). It contains many blood sinuses. The muscular portion, 80 microns thick, consists of closely intermingled circular and longitudinal fibers. The mesentery has the characteristics of the genus except in the mid-region of the body where a part of it is attached to the stalk.

The visceral mass is crowded with oocytes enclosed in thick-walled follicles of connective and muscular tissue. The walls of the follicles form a distinct network throughout the visceral mass and a few bands of muscle fibers pass transversely through it. In the lateral surfaces are embedded the 2 colleteric glands which protrude noticeably into the mantle cavity. Their canals are branched and arranged in 4 longitudinal rows. In sections where the largest number of canals occur the left colleteric gland has 64 and the right 52. The female genital pore in the mid-region of the gland has a cuticle 10 microns thick with spiny projections 16 to 20 microns in length.

The male reproductive organs are 2 large cylindrical sacs, nearly equal in size, located in the posterior portion of the visceral mass. Although the thick muscular walls of these organs are contiguous, their lumina do not fuse. The testes are strongly curved at the point of their abrupt transition into the vasa deferentia. The latter are long slender tubes surrounded by a muscular sheath. They take a somewhat tortuous course in their descent from the visceral mass to the mesentery and continue as separate, parallel tubes. A thick mass of chitin effectively obstructs the lumina of these vessels along their entire length. The vasa deferentia, as in *Loxothylacus panopaei*, do not open directly into the mantle cavity but into flattened vestibules which have a dorso-ventral length of 720 microns (Fig. 17). The walls of these cavities are 100 microns apart and have inwardly projecting folds. The vasa deferentia run parallel to the vestibules before entering them. Short, thick spines are present on the chitin of the vestibular walls, but where the vasa deferentia terminate and where the vestibules open into the mantle cavity larger spines occur, reaching a length of 20 microns.

Remarks: This new species has some characteristics in common with *Loxothylacus armatus* Boschma (1949) and *L. sclerothrix* Boschma (1933). These are the only known species of the genus that possess 30 or more spindles on their retinacula. The retinacula of *armatus* and *sclerothrix* differ slightly from those of *perarmatus* in having a flattened instead of a conical base. Although the external excrescences are also similar to those of *L. armatus*, the equal size of the testes, their very strong curvature, and the canals of the colleteric glands with 60 tubes arranged in four rows (*L. armatus* has more than 100 tubes) distinguishes this species from *armatus*. Moreover, the type locality of *L. armatus* is Talcahuano, Chile, while that of *L. sclerothrix* is the Western Indian Ocean. In the case of *L. sclerothrix* the testes are widely communicating at their terminal parts and the external excrescences are shorter and end in distal prominences.

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EXPLANATION OF PLATES

Abbreviations: *c.g.*, colleteric gland; *g.*, ganglion; *l.c.g.*, left colleteric gland; *l.t.*, left testis; *l.v.d.*, left vas deferens; *mes.*, mesentery; *m.op*, mantle opening; *st.*, stalk; *t.*, testis; *vb.*, vestibule.

Loxothylacus panopaei (Gissler)

FIGURE 1. Diagram of *Loxothylacus panopaei* viewed from the left side.
FIGURE 2. Excrescences on surface of external cuticle. Common type.
FIGURE 3. Excrescences on surface of external cuticle of a specimen found on *Panopeus herbstii*.
FIGURE 4. Type of excrescense with rounded tips.
FIGURE 5. Longitudinal section of parasite.
FIGURE 6. Part of longitudinal section at region of male genital openings.
FIGURE 7. Two retinacula found on internal cuticle.

Loxothylacus perarmatus, n. sp.

FIGURE 8. External appearance, viewed from the right side.
FIGURE 9. Surface view of external cuticle under high magnification.
FIGURE 10. Section through external cuticle showing excrescences and one of the pits containing a chitinous plug.
FIGURE 11. Surface view of chitinous plug embedded in external cuticle.
FIGURE 12. Excrescences of external cuticle from the left side of the animal.
FIGURE 13. Excrescences of external cuticle from the right side of the animal.
FIGURE 14. Section through the mantle showing folds projecting into the mantle cavity.
FIGURE 15. Two retinacula found on the internal cuticle.
FIGURE 16. Section through the colleteric gland.
FIGURE 17. Part of longitudinal section at region of male genital openings.

RESEARCH NOTE

THE OCCURRENCE OF *HEPATOZOON SCIURI* IN GRAY SQUIRRELS IN NEW ENGLAND

Herman and Price (1955, J. Protozool. **2**:48–51) reported the occurrence of a protozoan in the leucocytes of the gray squirrel, *Sciurus carolinensis*, from Maryland and the District of Columbia. Although they found it morphologically indistinguishable from *Hepatozoon muris*, a species found in rats (*Rattus norvegicus*), they have chosen to designate it tentatively as *Hepatozoon sciuri* Coles, 1914. In the same year Herman and Clark (1955, J. Parasitol. **41**(6, Sec. 2):17) predicted the possibility of a broad geographical distribution of *H. sciuri*.

During the Rhode Island Mammal Survey, blood was taken in November and December of 1956 from 11 squirrels collected from various areas of the state. Thin and thick blood films were made and stained with Giemsa stain. Subsequent study revealed the presence of parasites in the blood from 3 squirrels. These parasites compared favorably with those found on slides of squirrel blood kindly furnished the authors by Dr. C. M. Herman of the Patuxent Research Refuge, Laurel, Maryland. Since no morphological differences could be noted between the 2 series, the material from Rhode Island is herein considered conspecific with that identified as *Hepatozoon sciuri* by Herman and Price.

The finding of this parasite in squirrels in Rhode Island is the first evidence that the distribution of this parasite may be as broad as Herman and Clark predicted. These authors found that concentration techniques facilitated the finding of parasites and revealed a higher prevalence rate. No concentration techniques were used in the present study. If such techniques had been employed the number of infected squirrels may have been substantially increased.

This investigation was supported in part by a grant from the Federal Aid to Wildlife Act, Rhode Island Project W-6-R and W-23-R.—WILLIAM P. WEIDANZ AND K. E. HYLAND, *Departments of Bacteriology and Zoology, University of Rhode Island.*

PLATE I

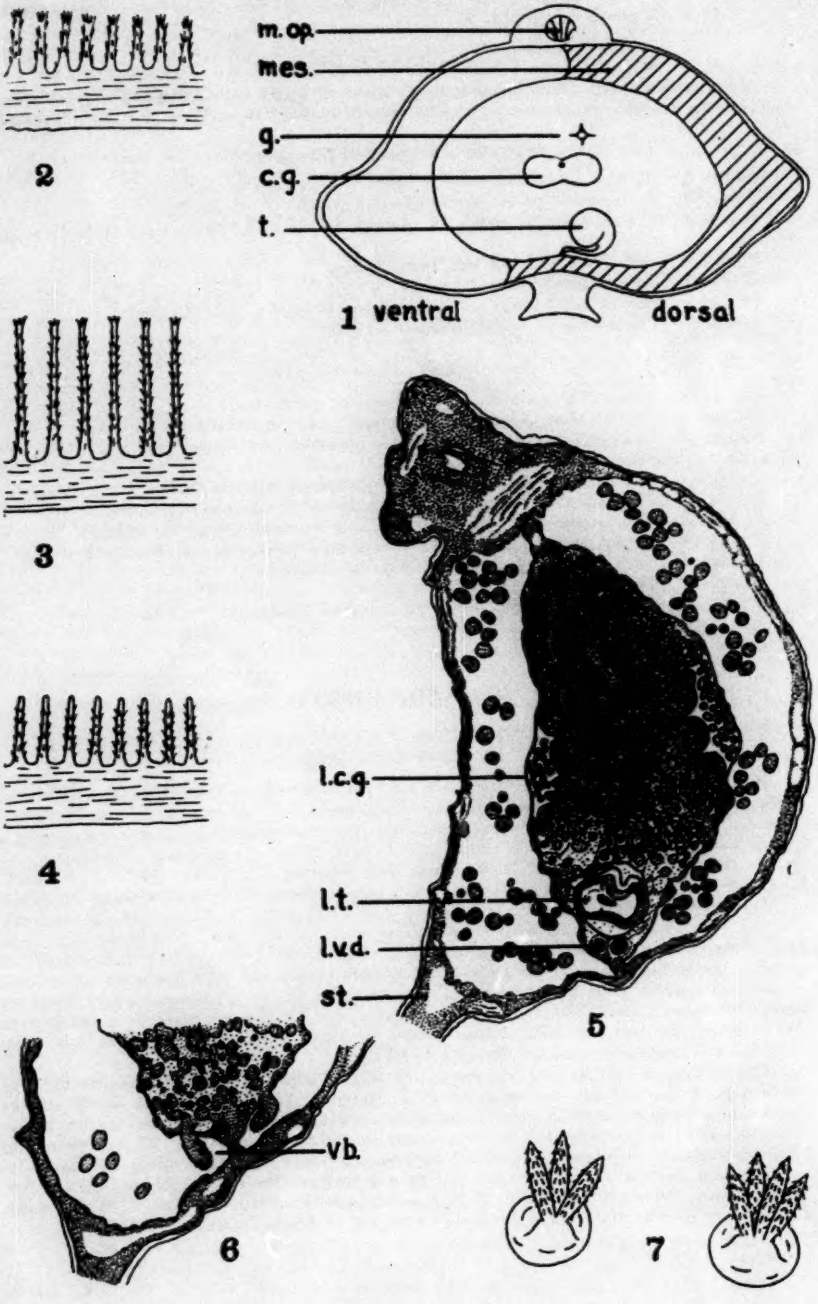
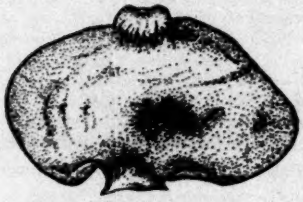


PLATE II



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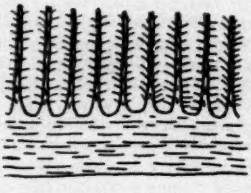

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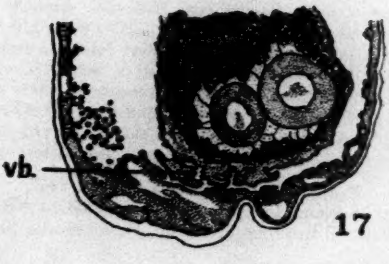

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CARNEOPHALLUS TURGIDUS SP. NOV. (TREMATODA: MICROPHALLIDAE) FROM THE RACCOON, *PROCYON LOTOR*, IN SOUTH FLORIDA

W. HENRY LEIGH
The University of Miami

Several thousand specimens of a small microphallid trematode were recovered from 2 raccoons from South Florida in February, 1956. Study of this trematode reveals it as a new species belonging to the genus *Carneophallus* established by Cable and Kuns (1951) to receive *C. trilobatus* from a Mexican Insect Hawk, *Buteo magnirostris griseocauda* and *C. pseudogonotylus* (Chen, 1944) from domestic ducks in China. Since then Coil (1956) has described *C. muellhaupti* from the Sanderling, *Crocethia alba*, from Mexico.

Cable's and Kuns' (1951) redefinition of the family Microphallidae Travassos, 1921 placed emphasis, in generic separation, on the presence or absence of a cirrus sac and the structure of the genital atrium and the male papilla. The species here described clearly fits Cable's and Kuns' description of the genus *Carneophallus* except for the presence of a seminal receptacle which was described as "apparently absent."

Morphology of this trematode was based on the study of living and fixed specimens. Details of the genital atrium, male papilla, excretory system, seminal receptacle and course of the uterus could be seen satisfactorily only in living material. Hot alcohol-formalin-acetic acid mixture was the fixative used and the stains were Ehrlich's acid hematoxylin and Semichon's carmine. Measurements were made on 20 fixed unflattened specimens.

Carneophallus turgidus sp. nov. (Figs. 1–4)
(Measurements in millimeters)

Diagnosis: with characters of the genus. Pyriform in shape with deep concavity posterior to intestinal ceca in unflattened specimens. Cuticular spination pronounced to the acetabulum becoming progressively reduced posteriorly; no spines posterior to testes. Body 0.280–0.368 in length (av. 0.335) and 0.207–0.257 in width (av. 0.231) at the testicular level. Oral sucker 0.048–0.058 in diameter (av. 0.055). Ventral sucker 0.038–0.058 in diameter (av. 0.047). Prepharynx lacking in all specimens examined. Pharynx longer than wide, measuring 0.019–0.029 in length (av. 0.026) and 0.016–0.029 in width (av. 0.023). Esophagus long and slender giving rise to ceca which end at level of ventral sucker; walls of ceca are thick except for small area at junction with esophagus. Testes regular in outline, usually transversely oval in shape measuring 0.035–0.058 in length (av. 0.041) and 0.042–0.077 in width (av. 0.060). Seminal vesicle with prostate gland located anterior to ventral sucker. Genital atrium large, thin-walled and located on left side. Male papilla consists of 3 lobes; ventral lobe is pyriform and partly covers 2 dorsal linguiform lobes whose bases overlap and are broadly joined to median wall of atrium. Course of ejaculatory duct and the exact point of its opening into genital atrium not determined. Genital pore somewhat variable in location but in area adjacent to left side of ventral sucker. Ovary, 0.051–0.070 in width (av. 0.062) by 0.035–0.058 in length (av. 0.047), anterior to right testis. Oviduct joined to right of median line by a common junction of vitelline ducts. Vitellaria consist of several ovoid masses on each side in testicular and posttesticular area. A seminal receptacle enters oviduct near entrance of common vitelline duct. Egg-filled uterus, not often extending anterior to the ventral sucker, runs from ovary to posterior end and back on right side before crossing to left side where it loops from posterior end to region of genital atrium and back before ascending again to enter atrium. Terminal part

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of uterus most often runs dorsal to genital atrium and enters it at an anterodorsal location after which it follows left side of atrium to open near middle of floor of that structure. Distal part of uterus equipped with a muscular ovijector and metraterm possesses a wrinkled cuticular lining. Eggs 0.014–0.020 in length (av. 0.017) by 0.008–0.010 in width (av. 0.009) usually obscure testes and vitellaria. Excretory bladder U-shaped; flame cell pattern 2[(2+2)+(2+2)] =16; excretory pore terminal.

Host: The Raccoon, *Procyon lotor.*

Locality: The Everglades region of South Florida.

Type specimens: Cotypes in the Helminthological Collection, U. S. National Museum No. 38269.

Carneophallus turgidus can be distinguished from *C. pseudogonotylus* (Chen, 1944) by the presence of 3 instead of 2 lobes in the male papilla. While *C. trilobatus* (Cable and Kuns, 1951) has a 3-lobed male papilla, the lobes are different in form and orientation from those of *C. turgidus.* The ventral lobe of *C. turgidus* is pyriform and its axis is at right angles to the other 2 lobes which are generally linguiform. The metraterm of *C. trilobatus* enters the genital atrium posterodorsally and medially while that of *C. turgidus* enters at an anterodorsal and lateral location, proceeds medially along the floor of the atrium and opens somewhere near the middle of that structure. *C. trilobatus* was described as having a prepharynx and a seminal receptacle was not mentioned. A prepharynx, present in most microphallid trematodes, was not seen in the several hundred specimens of *C. turgidus* examined; if present, it would be very short and seen only in an extremely extended condition. *C. turgidus* does possess a seminal receptacle visible, however, only in living specimens under pressure. The structure could not be seen in fixed and stained material. While not observed in the other 3 species of this genus, the seminal receptacle has been reported for other microphallids (Rankin, 1940; Coil, 1956). Cable and Kuns (1951) indicated that the presence of a seminal receptacle in the family Microphallidae was yet to be demonstrated and a fertilization chamber may have been misinterpreted as a seminal receptacle.

Carneophallus turgidis can be distinguished from the third species in the genus, *C. muellhaupti* (Coil, 1956) by the relatively light lobation of the male papilla of the latter, a condition more closely resembling that of *C. pseudogonotylus* (Chen, 1944).

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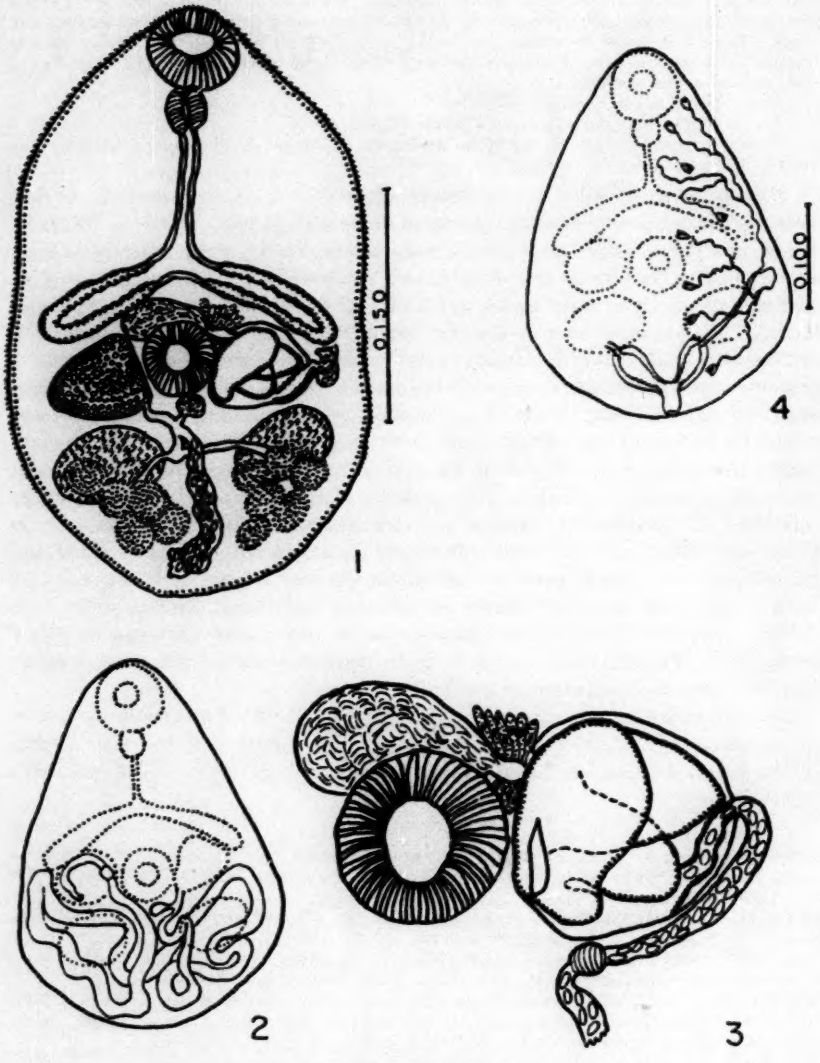
PLATE I



EXPLANATION OF PLATE

FIGURE 1. *Carneophallus turgidus* drawn with the camera lucida. Ventral view.
FIGURE 2. Outline drawing showing course of uterus. Dorsal view.
FIGURE 3. Enlarged sketch showing details of the genital atrium, male papilla, terminal part of uterus with ovijector and genital pore. Ventral view.
FIGURE 4. Outline drawing showing flame cell system.

A SUMMER SURVEY OF THE PARASITES OF TWO *MICROTUS PENNSYLVANICUS PENNSYLVANICUS* (ORD) POPULATIONS

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Some observations were made on the blood parasites of the field mouse, *Microtus pennsylvanicus pennsylvanicus* (Ord.), near Ithaca, New York (Tompkins County) as part of a mouse population study. The parasites were first noted in November 1953, when several mice from the field plots were examined in the laboratory in an effort to determine why they had lost weight. The data here presented were collected in the summer of 1954 to ascertain the incidence and possible effects of the parasites on the mouse populations. An abstract of this work has been published by Kirner, Barbehenn and Travis (1955).

STUDY PLOTS AND METHODS

The mice observed in these studies were from 2 field plots. The first plot of about 12 acres located half a mile east of the Tompkins County Airport included recently abandoned corn fields and an old pasture with wooded and open areas. Because the land had less than 1% slope and poor drainage, some standing water was present from October until late May. The wettest areas were densely covered with various sedges, wool grass, sensitive fern, and willows. The drier areas were covered with timothy, strawberry, hawkweed, cinquefoil and several species of goldenrod and asters. The second plot was a 1-acre field located 5 miles north of Ithaca. Exposure, drainage, and soil type were such that this area remained rather dry with no standing water. The plot was covered with a stand of uncut birdsfoot trefoil and orchard grass, with sedges in the wetter areas and with poverty grass on the dryer.

From June to August the mice were captured in live traps, with rolled oats and peanut butter used as bait. When the animals were removed from the trap they were toe-clipped for identification, smears made with blood from the wound, and the animals released. Smears taken from recaptured animals were obtained by docking the tail. In September, an attempt was made to capture all surviving mice in snap traps; liver smears were made from these dead animals.

The first plot was trapped between June 18 and July 1, July 27 and August 2, August 21 and 28, and September 17 to 26. The second plot was trapped from July 8 to 11, August 6 to 9, August 31 to September 2, and on September 26. In total, 175 mice were captured from plot 1 and 62 from plot 2.

RESULTS

Three parasites were observed in the blood smears of the mice, a sporozoan present in the erythrocytes, large numbers of rod-shaped bacteria present in individual erythrocytes, and a trypanosome. These were identified as *Babesia microtia* (Franca), *Haemobartonella microti* Tyzzer and Weinman, and *Trypanosoma microti* Laveran and Petit. The prevalence of the parasites in mice from the two plots varied considerably (Table I). The sporozoan was present in more than half the mice of the first plot, and completely absent from those of the second. The trypanosome prevalence varied between the two plots, but the differences were not significant. The bacterium was much more numerous in the second than in the first plot.

Infections of mice with any one parasite regardless of the presence or absence of one or both of the others, showed no significant change due to season (Table II).

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TABLE I.—*Percentage of mice infected with blood parasites in each of two plots*

Parasites	Plot 1 (175 mice)	Plot 2 (62 mice)
Babesia	63	0
Trypanosoma	22	10
Haemobartonella	6	34
No parasites	30	60

The observations in September were all made on dead animals and the parasites were particularly difficult to recognize. Perhaps as a consequence, the prevalences of parasites for September are lower than those of the other months. In the first plot the prevalence of *B. microtia* remained quite constant from June through

TABLE II.—*Percentage of mice of Plot 1, by months, infected with any of the 3 parasites regardless of presence of any other*

	June (51 mice)	July (65 mice)	August (95 mice)	September* (50 mice)
Babesia	53	52	54	44
Trypanosoma	20	12	20	6
Haemobartonella	8	0	0	0
No parasites	39	35	36	54

* Tissue smears from dead animals.

August, with an infection rate of about 50%, while trypanosomes were present in constant numbers at about half this rate, with their incidence dropping to about 6% in September. The bacterium disappeared after a low initial prevalence in June.

The effects of any one parasite upon the incidence of any other are shown in Table III. Only *T. microti* and *B. microtia* were found together with any regularity,

TABLE III.—*Percentage of mice, by months, infected with various combinations of 3 blood parasites in each of 2 plots. (Figure in parenthesis is the total number of mice examined)*

Parasites	Plot 1				Plot 2			
	June (51)	July (65)	Aug. (95)	Sept. (50)	July (30)	Aug. (42)	Sept. (8)	Sept.* (8)
Babesia alone (B)	35	49	39	38	0	0	0	0
Trypanosoma alone (T)	2	5	6	0	13	5	13	0
Haemobartonella alone (H)	6	3	4	2	20	31	25	38
B + T	16	8	14	6	0	0	0	0
B + H	0	0	1	0	0	0	0	0
T + H	0	0	0	0	0	0	0	0
B + T + H	2	0	0	0	0	0	0	0

* Tissue smears from dead animals.

and this association was observed only in the first plot. The prevalence of the sporozoan alone in this plot ranged from 35 to 49% from month to month, that of the trypanosome from 0 to 6%. However, the prevalence of double infection, sporozoan and trypanosome together in the same animal, which ranged from 6 to 16%, was sufficiently high to indicate some favorable interaction between the two parasites. The prevalence of the trypanosome was shown by the Chi square test to be significantly higher in the double infection than would be expected from random association. It also appears that some unfavorable interaction may have occurred between the trypanosome and the bacterium, for in the second plot where rather high prevalences of both was noted, no double infections were observed. In the first plot only 2 cases occurred with the bacterium in association, with one or the other, sporozoan or trypanosome; hence there seems to be unfavorable interaction between the two parasites.

When the data were subjected to Chi-square tests, no significant difference was indicated for the parasite prevalence in relation to the age and sex of the animals. In addition, there seemed to be no evidence that these parasites affected the survival of the mice.

SUMMARY

The seasonal incidence of 3 hemoparasites, *Babesia microtia* (Franca), *Trypanosoma microti* Laveran and Petit, and *Haemobartonella microti* Tyzzer and Weinman of the field mouse *Microtus pennsylvanicus pennsylvanicus* (Ord.) was studied from June to October, 1954 in 2 collecting areas at Ithaca, New York (Tompkins County). One area was wet with some standing water in the spring, and the other well drained and dry.

Sporozoan infections were most prevalent in the wet field, prevalence of the bacterium was highest in the dry field, and tended to diminish during the course of the study. The number of trypanosome infections tended to remain constant and equal in both areas. Some favorable interaction between the trypanosome and the sporozoan in multiple infections was observed, as well as a possible unfavorable interaction between the bacterium and the trypanosome and the bacterium and the sporozoan. There was no significant effect of the parasites upon mortality of the mice, nor was there any significant difference in rates of infection between sexes or among age groups.

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AMERICAN SOCIETY OF PARASITOLOGISTS

PRELIMINARY ANNOUNCEMENT OF THE THIRTY-THIRD ANNUAL MEETING

Sunday, Monday, Tuesday, and Wednesday,
August 24–27, 1958

Indiana University, Bloomington, Indiana

In accordance with the action of the Council, the American Society of Parasitologists will convene for a 4-day program in conjunction with the meeting of the American Institute of Biological Sciences.

The program will be arranged in the same manner as in previous years. The Council will meet at 2 P.M. on Sunday, August 24. Contributed papers to be read will be scheduled on Monday and Tuesday, August 25 and 26. The presidential address, annual luncheon, annual business meeting, and demonstration session will be scheduled on Wednesday, August 26.

The call for papers is being sent to members of the Society on March 15. In order that the program outline be available for the AIBS Bulletin and that the abstracts be printed and mailed to the membership before the meetings, it is necessary that requests for program space be received in the office of the Secretary not later than May 10, 1958. Members are hereby reminded of this date, and are requested to be prompt in the submission of papers for the program.

Information concerning registration, housing arrangements, food services, and an outline of the program will appear in the AIBS Bulletin.

PAUL E. THOMPSON
Secretary

TRAVTREMATINAE *NOM. NOV.* FOR LEPTOPHYLLINAE BYRD, PARKER AND REIBER, 1940 (PLAGIORCHIIDAE: TREMATODA)

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The genus *Leptophyllum* was established by Cohn (1902) for a trematode which he recovered from the intestine of the South American snake, *Herpetodryas* (= *Chironius*) *fuscus*. Ruiz and Leão (1942) pointed out that Cohn's *Leptophyllum stenocotyle* (Fig. 1) and *Travtrema travtrema* Pereira (1929) from *Xenodon* (= *Liophis*) *merremii* (Wagler) (Fig. 4) were probably synonyms. Pereira's material, for which he (1929) established the new genus, *Travtrema*, was collected at the Instituto Butantan, Brazil. Since that time additional species described include *L. tamiamiensis* McIntosh, 1939, from *Agkistrodon piscivorus* from Florida (Fig. 3) and *L. ovalis* Byrd and Roudabush, 1939, from *Natrix taxispilota*, purchased from Florida (Fig. 2).

Ruiz and Leão (1942) obtained material from 5 species of South American snakes during a survey of this genus and found such wide individual variation that they were inclined to place them all in the species *L. stenocotyle* Cohn, 1902.

Schad (1953) obtained a series of *Leptophyllum* from the snake, *Elaphe quadrivittata deckerti*, and found no consistent differences between his specimens and both *L. tamiamiensis* and *L. ovalis*. His conclusion, also, was that *L. ovalis* and *L. tamiamiensis* were probably synonymous.

Both Cohn and McIntosh suggested that *Leptophyllum* showed relationship to *Enodiotrema* Looss, 1900. McIntosh (1939) placed *Leptophyllum* in the Enodiotrematinae Baer, 1924. Byrd, Parker and Reiber (1940) studied the excretory system of both *Leptophyllum* and *Paurophyllum* Byrd, Parker and Reiber, 1940, and came to the conclusion that both genera are members of the Plagiorchiidae Ward, 1917. On this basis they established for these two genera the subfamily Leptophyllinae. Ruiz and Leão (1942) were noncommittal about the validity of the subfamily Leptophyllinae but placed *Leptophyllum* and *Paurophyllum* in the family Plagiorchiidae.

Hughes, Higginbotham and Clary (1942) retained the species *L. stenocotyle*, *L. tamiamiensis* and *L. ovalis* in the Plagiorchiidae, but were uncertain about the proper placement of *Travtrema travtrema* Pereira, 1929, listing it as "subfamily undetermined."

In 1947, upon recovery of 1 mature and several immature specimens of the genus *Leptophyllum* from the large intestine of the Mud Snake, *Farancia abacura abacura* Holbrook from Florida, it was found that *Leptophyllum* is not available as a generic name for trematodes. Verhoeff (1895) used the name *Leptophyllum* as a genus of Myriapoda, Diplopoda, thus making *Leptophyllum* Cohn, 1902 a homonym of *Leptophyllum* Verhoeff, 1895. Fortunately, the name *Travtrema* Pereira, 1929 is available for *Leptophyllum* (Trematoda), the new designation being *Travtrema stenocotyle* (Cohn, 1902) *comb. nov.*, with *Travtrema travtrema* Pereira, 1929, *T. tamiamiensis* (McIntosh, 1939) *comb. nov.* and *T. ovalis* (Byrd and Roudabush, 1939) *comb. nov.* as probable synonyms.

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With the reëstablishment of *Travtrema* Pereira, 1929 for this group of trematodes it becomes necessary to establish for Leptophyllinae the new subfamily name, Travtrematinae. As the limits of the subfamily Travtrematinae are the same as those of Leptophyllinae the subfamily diagnosis of Byrd, Parker and Reiber (1940) for Leptophyllinae will be used with but slight changes.

subf. TRAVTREMATINAE *nom. nov.*

Subfamily diagnosis: Small, distomate flukes, with or without cuticular spines, suckers equal or subequal, with the ventral sucker in, or anterior to, the body middle; digestive tract consisting of pre-pharynx, muscular pharynx surrounded by gland cells, short esophagus, and tubular ceca that extend back to or beyond gonads into posterior part of body; genital pore to one side of midline, midway between bifurcation of ceca and ventral sucker; cirrus sac moderately stout, curving around ventral sucker to near its caudal boundary, containing dilated, coiled *vesicula seminalis*, slightly muscular *pars prostatica*, short ductus, and muscular cirrus; cirrus armed (*Travtrema*) or unarmed (*Paurophyllum*), eversible; metraterm powerfully developed, muscular, with peri-vaginal gland cells, about as long or longer than cirrus sac; ovary to one side of midline at end of cirrus sac, smaller than testes; *receptaculum seminis* large, behind ovary; oötype, Mehlis gland, and Laurer's canal present; uterus descending and ascending between testes, descending to near posterior margin of body; ova numerous, operculated, fully embryonated when oviposited; vitellaria follicular, mainly lateral to ceca in region of ovary; testes close behind ovary, equal or subequal, opposite or oblique, with smooth to irregular borders; excretory bladder I-shaped, with long stem, giving rise to common collecting tubules close behind level of ovary; common tubules passing upward to level of anterior margin of acetabulum before branching into anterior and posterior collecting tubules; flame cell formula 2 [(3+3+3) + (3+3+3)] = 36. Parasitic in digestive, reproductive, and excretory systems of snakes. Type genus: *Travtrema* Pereira, 1939. Additional genus, *Paurophyllum* Byrd, Parker and Reiber, 1940.

The type and probably only species of *Travtrema is T. stenocotyle* (Cohn, 1902) *comb. nov.*, while the genus *Paurophyllum* contains the 2 species, *P. simplexus* and *P. megametricus*, both Byrd, Parker and Reiber, 1940.

SUMMARY

1. *Leptophyllum* Cohn, 1902 (Trematoda) is a homonym of *Leptophyllum* Verhoeff, 1895 (Myriapoda).

2. *Travtrema* Pereira, 1929 is available for *L. stenocotyle* Cohn, 1902, the name becoming *Travtrema stenocotyle* (Cohn, 1902) *comb. nov.*

3. The subfamily name Travtrematinae is used to replace Leptophyllinae for this group of trematodes. This subfamily includes the genera *Travtrema* Pereira, 1929 and *Paurophyllum* Byrd, Parker and Reiber, 1940.

ACKNOWLEDGMENT

I wish to thank Dr. George R. La Rue for reading the manuscript and for his counsel and guidance when he was chairman of the author's doctoral committee at the University of Michigan.

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EXPLANATION OF FIGURES

FIGURE 1. *Travtrema stenocotyle* (Cohn, 1902).
FIGURE 2. *Travtrema ovalis* (Byrd and Roudabush, 1939).
FIGURE 3. *Travtrema tamiamiensis* (McIntosh, 1939).
FIGURE 4. *Travtrema travtrema* Pereira, 1929.

RESEARCH NOTE

SWIMMER'S ITCH IN NEW JERSEY

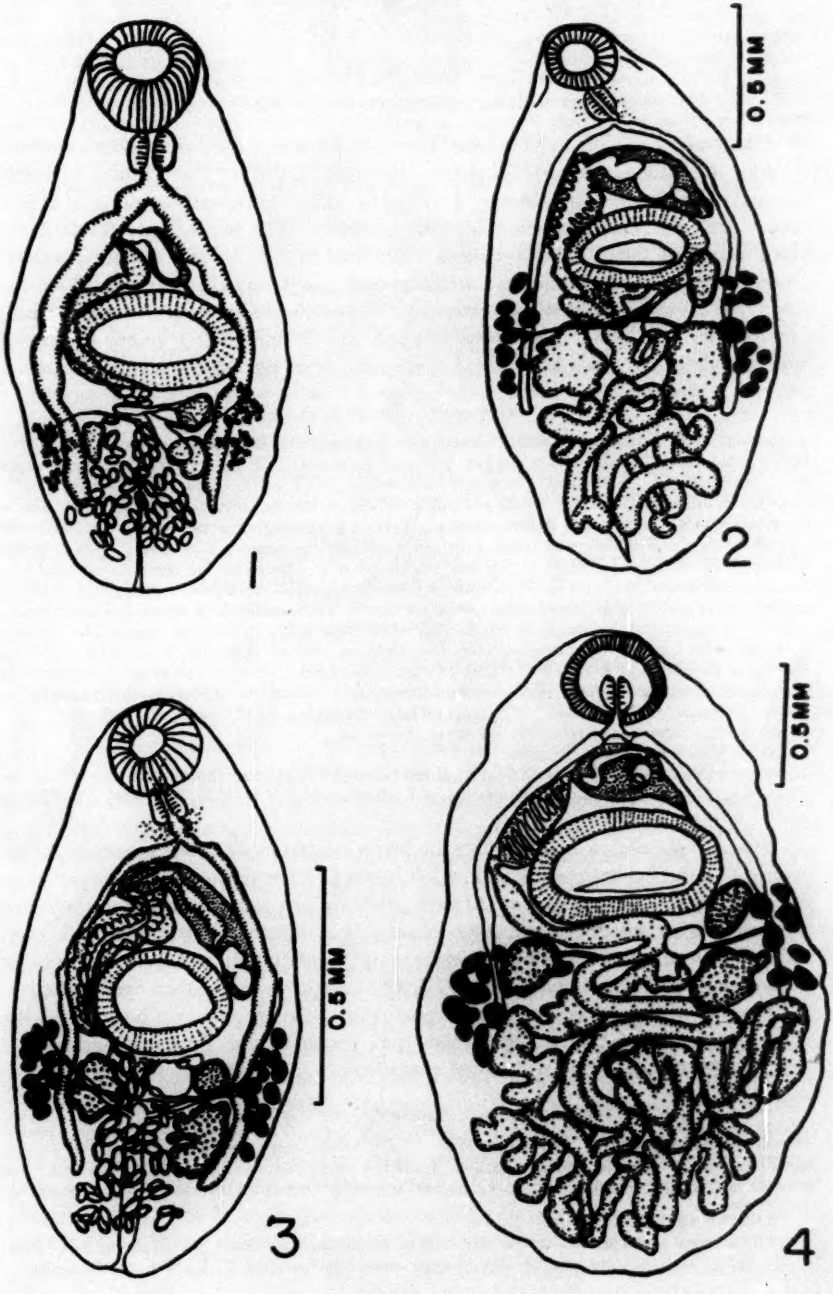
Swimmer's itch, or cercarial dermatitis, has been reported in the literature from many States since the early reports of Cort in the late 1920's. To the writer's knowledge, none of these reports includes New Jersey. Dermatitis-producing cercariae emerging from both fresh water and marine snails are now well recognized and the list of new irritant species is still being lengthened. This note reports the occurrence of the first human cases of cercarial dermatitis for New Jersey and, furthermore, records cases associated both with fresh and marine waters. The 2 sets of cases both occurred in the summer of 1957.

The cases associated with fresh water all appeared from contact with a swimming pond in Summit, New Jersey. The pool, a dammed portion of a spring-fed brook, was constructed about a year previously. Wild ducks had been observed on it and the family kept domesticated ducks on it as well. In June, 1957 the family and friends using the pond noted episodes of skin eruption, clearly associated them with entry into the water, and recognized that on cloudy days they were not "bitten" so often as on warm bright sunny days. Through the cooperation of the assistant county agricultural agent of Union County, Raymond N. Eberhardt and Dr. Robert E. Treece, extension entomologist at the Rutgers-State University-College of Agriculture, to whose attention the problem was brought, snails were obtained from the pond for laboratory study. Infection was observed in 1 of the snails, kindly identified by Dr. Henry van der Schalie, Museum of Zoology, University of Michigan as *Physa heterostropha*. The cercariae most closely resembled those of *Cercaria physellae*. The species of snail and of cercaria, therefore, are identical with those reported in the New York outbreak (Holla, W. A. and Lands, E. A., 1947. N.Y. State J. Med. **49**: 290–302). The writer witnessed maculeformation on one member of the family soon after exposure.

The author's children and his own person were involved in the episode of "clam digger's itch" from marine waters. We had gone out to dig clams on the tidal flats of the Bay Shore of Cape May near Dias Creek just before low water when the water was but a few inches deep. In the presumably previously unexposed children the lesions were delayed in appearance, itched much less and faded sooner than in the author, both of whose feet were liberally covered with lesions. This is an interesting point because, although the author had collected and swum literally hundreds of times since early childhood in several areas of New Jersey waters where infected snails have been found by him or his students (McDermott, J. J. 1951, Unpublished Master's thesis, Rutgers U.) this was his first known instance of infection. Nevertheless, his immediate response as a sensitized individual suggests previous but unknown sensitization by cercariae. Collections of the suspected snail, *Nassarius obsoletus*, yielded infections with *Microbilharzia variglandis*, a cercaria known to be dermatitis-producing (Stunkard, H. W. and Hinchliffe, M. C. 1952. J. Parasitol. **38**: 248–265).

Cercarial dermatitis has probably been present in New Jersey sporadically for years and there have been suggestive but unconfirmed reports to support this. In tidal waters it appears that unusual circumstances may be necessary for infection to take place. On the other hand, it is probable, with the increase in the number of artificial ponds for the purposes of swimming and irrigation, that further outbreaks of swimmer's itch will be noticed.—LESLIE A. STAUBER. *Rutgers, The State University, New Brunswick, New Jersey.*

PLATE I



A REVISION OF THE GENUS *GYNAECOTYLA* (MICROPHALLIDAE: TREMATODA) WITH A DESCRIPTION OF *GYNAECOTYLA RIGGINI* N. SP.*

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Five mature specimens of a small microphallid trematode were recovered from a ruddy turnstone, *Arenaria interpres morinella*, taken at Bald Point, Franklin County, Florida, by Mr. George T. Riggin. They apparently represent a new species and are named in honor of their collector. The genus *Gynaecotyla* is revised to include them. The specimens were fixed in A.F.A., under slight pressure. Three were stained in Semichon's aceto-carmine and mounted in neutralized damar. The other 2 were imbedded and sectioned. The sectioned material was stained with hematoxylin and eosin. All measurements are in millimeters unless otherwise stated, and the averages are from 3 specimens, with the ranges in parentheses.

Gynaecotyla riggini n. sp.

Diagnosis: With characters of genus *Gynaecotyla* (*vide infra*). Body small, elongate-oval, 0.86 × 0.33 (0.66–1.00 × 0.29–0.35). Oral sucker 0.05 dia. (0.04–0.06). Two acetabula present at same level, both 0.05 dia. (0.04–0.06). Prepharynx 0.04 long (0.02–0.07). Pharynx 0.04 long (0.039–0.044). Esophagus 0.1 long. Ceca extending postero-laterally, then turning medially almost to anterior edge of testes. Testes opposite, weakly to strongly lobed, measuring 0.12 × 0.08 (0.058–0.109 × 0.097–0.146) with long axes perpendicular to long axis of body. Cirrus pouch 0.19 long (0.163–0.238). Male copulatory organ *sinistral*, consisting of complicated muscular structure composed of 2 curved, spatulate projections covered with chitin and lying in atrial cavity. Prostate weakly developed. Ovary smooth, retort-shaped, anterior to right testis, lateral to right acetabulum, 0.09 × 0.08 (0.06–0.09 × 0.05–0.08). Seminal receptacle present. Laurer's canal opening on dorsal surface, just right of midline posterior to vitelline reservoir. Vitellaria composed of 7–9 large follicles in each lateral field, anterior to testes; vitelline ducts uniting near midline to form small vitelline reservoir. Uterus extensively coiled posterior to testes. Metraterm thin-walled. Eggs operculate, measuring 0.018 × 0.010 (Av. of 25).
Host: Ruddy turnstone— *Arenaria interpres morinella.*
Locality: Franklin County, Florida.
Holotype: Deposited in Helminthological Collection, U.S.N.M. Col. No. 38246.
Paratypes: Deposited in the Helminthological Collection, U.S.N.M. Col. No. 38246 and Florida State University Collection.

Gynaecotyla riggini n. sp. differs in 3 respects from all other members of the genus: (1) the genital pore is on the left instead of the right, (2) the ovary is on the right instead of the left, and (3) the vitellaria are anterior to the testes. The presence and/or amount of testicular lobation is so influenced by pressure (Ulmer, 1952) that in the specimens of *G. riggini* n. sp. the condition is considered to be of little or no taxonomic importance. It is the author's opinion that the reversal in the position of the ovary and genital pore and the more anterior position of the vitellaria are not of generic significance and, therefore, the generic description is emended to include forms with these characters.

Genus *Gynaecotyla* Yamaguti, 1939, *char. emend.*

Diagnosis: Microphallidae Travassos, 1921. Very small, pear-shaped to elongate-oval with spiny cuticula; 2 ventral suckers located in middle body third. Prepharynx, pharynx, and esophagus present; intestinal crura extending to region between anterior edge of acetabula

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* This work was done under the direction of Dr. Robert B. Short. The author is grateful to Mr. Allen McIntosh for prompt loan of type specimens from the U. S. National Museum.

and anterior edge of testes. Genital pore dextral or sinistral. Male copulatory organ, complicated, muscular, lying in genital atrium, composed of 2 curved, horn-like or spatulate projections, usually with chitinized and armed tips. Large transverse cirrus pouch anterior to ventral suckers, containing seminal vesicle, ductus ejaculatorius, and weak pars prostatica. Ovary dextral or sinistral, always on side of worm opposite genital pore, and at level of ventral suckers. Testes symmetrical, posterior to ventral suckers, smooth or lobed. Uterine coils filling space behind testes. Lauer's canal and receptaculum seminis present. Vitellaria follicular, in 2 compact groups, in lateral fields posterior to ventral suckers and anterior or posterior to testes. Excretory bladder V-shaped; flame cell pattern, [2(2+2) + (2+2)]. Adults in intestine and ceca of shore birds; metacercariae in crustaceans.

Type species: Gynaecotyla squatarolae (Yamaguti, 1934) Yamaguti, 1939. Other species are:

G. riggini n. sp.

G. simillimus (Travassos, 1921) Rankin, 1940

Syn: *Levinseniella simillimus* Travassos, 1921; *Cornucopula simillimus* (Travassos, 1921) Rankin, 1939

G. adunca (Linton, 1905) Rankin, 1940

Syn: *Distomum aduncum* Linton, 1905; *Levinseniella adunca* (Linton, 1905) Linton, 1928; *Cornucopula sippiwissettensis* Rankin, 1939; *Cornucopula nassicola* (Cable and Hunninen, 1938) Rankin, 1939; *Gynaecotyla nassicola* (Cable and Hunninen, 1938) Rankin, 1940; *Gynaecotyla jägerskioldi* (Travassos, 1921) Rankin, 1940; *Levinseniella jägerskioldi* Travassos, 1921; *Cornucopula jägerskioldi* (Travassos, 1921) Rankin, 1939.

It should be noted that Hunter and Vernberg (1953) reduced *G. nassicola* to synonomy with *G. adunca* because the size varies greatly with the host, and the degree of folding of the intestinal crura depends upon the state of contraction of the worms. As the characteristics of *G. jägerskioldi* are intermediate between those of *G. adunca* and *G. nassicola*, it follows that, if *G. nassicola* is synonymous with *G. adunca*, *G. jägerskioldi* is also synonomous with *G. adunca*.

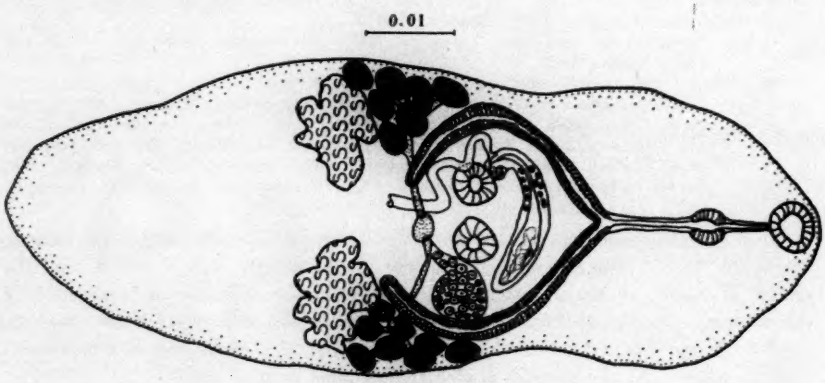
There has been some discussion as to the systematic position of the genus *Gynaecotyla*. Hunter and Vernberg (1953) considered it extremely doubtful that the genus *Gynaecotyla* belongs in the family Microphallidae on the following grounds: (1) the extreme modification of the genital atrium; (2) the reversed position of the genital atrium; (3) the presence of 2 acetabula; and (4) the life cycle as reported by them. However, (1) the copulatory complex of microphallid trematodes is an especially plastic one (Cable and Kuns, 1951); (2) *G. riggini* n. sp. has all the characteristics of the other species of *Gynaecotyla*, except that the position of the genital structures is not reversed; (3) the genus *Microphalloides*, which is apparently closely related to *Gynaecotyla*, has only 1 acetabulum; and, (4) Hunter and Vernberg (1956) pointed out that the cercaria they had earlier described (1953) as the larval stage of *G. adunca* was in reality the larva of *Zoogonus rubellus*. Therefore, for the present, there seems to be no reason to question the validity of placing *Gynaecotyla* in the family Microphallidae.

In another paper, (in press), the author points out that he considers the genus *Microphalloides* valid, at least until the type species is restudied, and as a possible intermediate stage in the evolution of the genus *Gynaecotyla*. Cable and Kuns (1951) believed that *Microphalloides* had a sinistral genital pore and placed it in the main line of evolution of their phylogenetic chart. Yoshida (1917) originally described a metacercaria as having a sinistral genital pore; but later, after stating that his original description was in error in regard to the position of the genital pore, he (1938) described a new genus, *Microphalloides*, characterized by a dextral genital pore. Then he made the adult stage of his previously described metacercaria the type of this genus. Therefore, since *Microphalloides* has a dextral genital pore and chitin associated with the male copulatory organ, it appears more logical

to place it in a position intermediate between the main line of evolution and the genus *Gynaecotyla*, rather than in the main line itself.

A Key to the Species of *Gynaecotyla*

1. a. Ovary dextral ..... *G. riggini* n. sp.
   b. Ovary sinistral ..... 2.
2. a. Ventral suckers differing by more than 0.02 mm in diameter ..... *G. simillimus*
   b. Ventral suckers not differing by more than 0.02 mm in diameter ..... 3.
3. a. 10 or more vitelline follicles on each side ..... *G. squatarolae*
   b. Less than 10 vitelline follicles per side ..... *G. adunca*



EXPLANATION OF PLATE

Gynaecotyla riggini n. sp.

Dorsal view, drawn with the aid of a camera lucida. Scale in millimeters. Most of the uterus not drawn.

SUMMARY

Gynaecotyla riggini is described as a new species and the characters of the genus are emended to include it. The phylogenetic relationship of the genus *Gynaecotyla* with the other genera in the family Microphallidae is discussed. It is concluded that, for the present, the genus *Gynaecotyla* should be retained in the Microphallidae. *G. jägerskioldi* is reduced to synonomy with *G. adunca*. A key to the species of the genus *Gynaecotyla* is presented.

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SOCIETY BUSINESS

AMERICAN SOCIETY OF PARASITOLOGISTS

Forty-Seventh Council Meeting, Philadelphia, Pennsylvania, October 30, 1957

The meeting of the Council of the American Society of Parasitologists was called to order by President Gilbert F. Otto at 7:45 P.M., Wednesday, October 30, 1957, in the Lafayette Room, Benjamin Franklin Hotel, Philadelphia, Pennsylvania. In addition to President Otto, the meeting was attended by President-Elect A. C. Walton, Vice-President A. McIntosh, Past Presidents H. E. Meleney and H. W. Stunkard, Treasurer R. M. Stabler, the Secretary, Councilors W. S. Bailey (ex-officio), P. C. Beaver, R. M. Cable, A. O. Foster, L. Jacobs (ex officio), F. J. Kruidenier, D. V. Moore, L. O. Nolf, and L. A. Stauber, and Committee Chairmen C. M. Herman, K. C. Kates, N. D. Levine, and L. J. Olivier. R. B. Hill, Secretary of the American Society of Tropical Medicine and Hygiene, was present as an observer. G. H. Ball attended the portion of the meeting concerned with his report.

I. Reports of Officers and Members of the Council

1. *President (G. F. Otto):* The President expressed his appreciation to the Officers, Councilors, and Committee Representatives for their efforts during the current year in behalf of the Society.

2. *President-Elect (A. C. Walton):* No report.

3. *Vice-President (A. McIntosh):* No report except to thank the members of the Council for indicating promptly their reaction to the proposal of a joint Symposium with the American Society of Tropical Medicine and Hygiene.

4. *Secretary (P. E. Thompson):* As of October 29, 1957, there were 994 members of the Society. Of these, 7 are non-subscribing members, 3 are Life Members, 10 are Foreign Honorary Members, and 81 are delinquent for one or more year's dues. During the past year, 10 members have died, 7 members have resigned, 15 people have been dropped for non-payment of dues, and 69 new members have been elected. The names of 9 applicants for membership are to be presented for Council action during this meeting.

Special problems handled by this office during the year include: (1) Obtained official recognition of the Society as a tax-exempt organization by the Bureau of Internal Revenue; (2) The A.I.B.S. addressograph records of Society members were brought up to date and a plan was instituted to keep this list current so that the A.I.B.S. addressograph services may be used effectively; (3) Copies of the proposed new constitution were reproduced and sent to the members of the Society; (4) A list of the members was prepared for publication in the 1957 Supplement of the Journal of Parasitology; (5) Supplied a list of the members and their addresses to the Secretary of the Sixth International Congress of Tropical Medicine and Malaria.

The report was accepted and ordered placed on file.

5. *Treasurer (R. M. Stabler):* The report submitted for the year November 1, 1956 to November 1, 1957 is summarized as follows:

Balance on Hand, November 1, 1956	$ 3,415.62
Collections to November 1, 1957	15,302.75
Total Funds for 1957	$18,718.37
Total Expenditures for 1957	15,239.23
Total Cash Balance November 1, 1957	$ 3,479.14

In submitting his report, the Treasurer pointed out that the Society has operated at a deficit of approximately one thousand dollars annually during the past two years (bills amounting to approximately $1200.00 are yet to be paid out of the November 1, 1957 balance).

The report was gratefully accepted subject to the report of the Auditing Committee and ordered placed on file.

6. *Custodian of Back Issues (W. S. Bailey):* The report submitted for the period November 1, 1956–September 30, 1957 is summarized as follows:

Balance on Hand, November 1, 1956	$1684.13
Net Collections During Period	$3564.54
Expenditures During Period	$1078.09
Balance on Hand, September 30, 1957	$4170.58

The total sales of back issues increased sharply during the past year: $4397.49 for 1957, $1885.45 for 1956, and $1978.70 for 1955.

The report was gratefully accepted subject to the report of the Auditing Committee and ordered placed on file.

7. *Custodian of Endowment Fund (N. R. Stoll):* This report covers the period August 15, 1956–October 31, 1957.

	1956	Interest earned	1957
Savings Account*	$ 475.26	$14.36	$ 489.62
U. S. Savings Bond†	$ 835.00	$26.00	$ 861.00
	$1310.26	$40.36	$1350.62

* No. 5197, Princeton Savings and Loan Assn.
† No. M1 681 662F of $1000.00, issued January, 1950 at purchase price of $740.00.

The report, having been certified by William Trager and Ira Singer, was accepted and ordered placed on file.

8. *Chairman of the Editorial Committee (L. Jacobs):* Owing primarily to an 8% increase in printer's rates, the cost to the Society per page of the Journal of Parasitology has increased from $15.61 in Volume 42 (1956) to $16.30 in Volume 43 (1957), with the prorated cost of the Volume 43 Index yet to be included.

Advertising rates were increased during the year to partially offset rising costs; so far, this has induced no resistance from patrons.

An analysis of the subject matter shows that helminthology occupies 54.4% as compared to 61.6% last year, entomology occupies about the same space (18.9% *versus* 20.8%), and protozoology occupies 20.4% as compared to 6.2% last year. Among the papers on helminthology, 44% were strictly taxonomic in character while 56% presented observational or experimental data. These distributions seem to represent an appropriate balance for a Journal representing all branches of parasitology.

A total of 306 papers was received during the period December 1955 through September 1957; 58% were accepted with little or no modification; 38% were returned for revision; 4% were rejected. The general practice is to obtain the opinion of two reviewers on each paper.

The report was gratefully accepted and ordered placed on file.

(Following the presentation of his formal report, Dr. Jacobs informed the Council that he wished to resign from his position as Chairman of the Editorial Committee effective at the end of the calendar year 1958. However, he mentioned that he might be able to continue if the contemplated A.I.B.S. services materially reduced the burden of the office.)

II. Reports of Standing Committees

1. *Committee on Visual Education and Instruction (M. S. Ferguson, Chr., D. V. Moore, J. C. Swartzwelder):* Requests for information on the availability of parasitological films have continued to be received and answered. Several motion pictures not previously included in our list have come to our attention. Since the supply of the compilation "Visual Aids to Instruction in Parasitology" is almost exhausted, and since it should be brought up to date, a revision of this material is underway. Arrangements have been made for the multilithing of the new list in an attractive and conveniently-sized booklet. Copies should be available for mailing to the membership by the first of the year.

The report was accepted and ordered placed on file.

2. *Committee on Nomenclature and Terminology (A. McIntosh, Chr., G. W. Hunter III, F. J. Kruidenier, G. W. Wharton; N. M. Stoll, Associate):* The resolution adopted by the Council during its 46th meeting objecting to including parasites of the "Collective Group" under the "parataxa plan" was published September 11, 1957 in Volume 15 (Part 1/4) of the *Bulletin of Zoological Nomenclature.* A similar objection was expressed also by a large majority of the 41 persons, including the Chairman, that attended the meeting of the Nomenclature Discussion Group of Washington, D. C., on August 7, 1957.

The report was accepted and ordered placed on file.

3. *In Memoriam Committee (M. A. Doss, Chr., E. Chernin, M. Voge):* The Committee noted the names of 10 members of the Society who have passed away since the 1956 report of the Committee. These names appear on page 53 of the Annual Supplement and Program of 1957.

The report was accepted with a deep sense of loss to the Society and ordered placed on file.

III. Reports of Representatives and Delegates

1. *To Council of the A.A.A.S. (R. M. Cable, R. P. Hall):* Council voted to endorse merger of *Science* and *Scientific Monthly* as proposed by the Board of Directors, Editorial Board, Committee on Publications and the Association's Staff. The merger, if undertaken, would depend on financial prospects and would not go into effect before January, 1958. The major increase

in the Association's activities during recent years has been toward improving science teaching. Substantial gifts and grants toward that end were listed in the Annual Report of the Executive Director. The Constitution was amended to eliminate the category of "associate" as applied to other organizations and Council voted to change the status of all organization including A.I.B.S. previously listed as associates to the status of affiliates. Eight new affiliates were elected. The Board recommended that no further action be taken regarding the proposal that affiliated organizations be assessed $25 per year per Council member to partly reimburse travelling expense of members attending meetings of the Council. Reaction of affiliated societies to the proposal was predominantly unfavorable. Council took no action on the recommendation. A lengthy interim report of the Committee on the Social Aspects of Science was distributed in advance of the New York meetings. A resolution that the Committee be enlarged and its work continued was approved by vote of Council.

Presented before Council by the Resolutions Committees were:

a. A resolution concerning fellow scientists in Hungary. Adopted by vote of Council.

b. A resolution concerning federal aid and freedom of research and teaching. Referred back to the Committee with instruction to make further recommendations to the Board of Directors.

c. A recommendation by the Committee that a resolution received by the Committee and concerning participation by biologists in the International Geophysical Year be referred to A.I.B.S. Recommendation of Committee sustained by Council after debate.

The report was accepted and ordered placed on file.

2. *To Governing Board of A.I.B.S.* (*A. C. Walton; L. J. Olivier,* alternate): The Governing Board of the American Institute of Biological Sciences met August 24, 1957, on the campus of Stanford University, Palo Alto, California, with 24 of the member societies represented.

Reports of the various officers and standing committees were presented and accepted after appropriate discussion. The Treasurer pointed out that the budget for the coming year would run in the vicinity of $350,000 due to the large number of grants to be administered by the Institute. Of this amount only a very moderate sum was to come from the dues of member Societies. The Executive Secretary reviewed the general program of the past year and emphasized (1) the success of the Visiting Biologists Program which served about 70 colleges and high schools and plans for extension of this service during the coming year (28 lecturers participated in this activity); (2) the Placement Service was successfully active and offered 125 positions to 185 applicants (there are now over 125 registrants and over 60 institutions listing vacancies with the Placement Bureau; (3) better placement of Biologists by the Armed Services has been attained through the membership of the A.I.B.S. on the Scientific Man Power Commission; (4) the National Register of Scientific and Technical Personnel now contains the names of over 60,000 biologists—names obtained largely through the efforts of A.I.B.S.; (5) the increasing activity of the A.I.B.S. in serving as an intermediary between individuals wishing to attend International Congresses and agencies willing to support travel to such meetings; (6) the increased importance of the Advisory Committee of A.I.B.S. in its service to various Governmental agencies; (7) the sponsoring of various Conferences and Symposia in addition to the Annual Meeting; (8) an increased coverage of Russian Journals to be translated; and (9) the acquisition of the Quarterly Journal of Biology, which will be available at a reduced rate to members of our Society as a result of its affiliation with the A.I.B.S.

Because of the increasing activities, it was voted to hold two meetings per year of the Governing Board instead of the present one. An early spring meeting is to be held in addition to the annual summer gathering.

The present officers, President Fenn, Vice President Kramer, and Treasurer Cullinan, were re-elected for 1958, as was Executive Secretary Cox.

The present plans for future meetings are as follows: 1958, Bloomington, Indiana; 1959, Penn State, Pennsylvania; 1960, Oklahoma State, Oklahoma; 1961 and 1962 open; 1963, University of Massachusetts.

The report was accepted and ordered placed on file.

3. *To Division of Biology and Agriculture of the National Research Council* (*N. D. Levine; W. S. Bailey,* alternate): The annual meeting of the Division was held at Washington, D. C., on May 3-4, 1957, with 45 persons attending and L. A. Maynard, Cornell University, presiding.

The booklet, "Career Opportunities in Biology", of which 30,000 copies were printed and distributed to high schools last year, is now out of print. It will be reprinted. A booklet on concepts in biology is in preparation. A booklet on high school biology, incorporating material from 130 high school and college teachers, is being prepared for early publication. A booklet, "Improving College Biology Teaching", is ready for publication. Copies will be sent to biology departments and college and university administrators. A "Sourcebook of Laboratory and Field Studies for Secondary School Biology Courses" is to be prepared this summer, and will

be tested in selected high schools during 1957–58. A report on the conference on undergraduate curricula in the biological sciences which was held in Washington December 8–9, 1956 and Chapel Hill, N. C. April 1–4, 1957 will be published in about 2 months. Two *ad hoc* panels to consider ways for improving undergraduate courses in specific subjects are working in the areas of systematic botany and parasitism. They are expected to report this summer. The panel on parasitism courses is composed of Drs. Clay G. Huff (chairman), L. O. Nolf, R. J. Porter, Clark P. Read, Glenn A. Richards and Leslie A. Stauber. Dr. M. H. Trytten of the Office of Scientific Personnel reported on Hungarian refugee scientists. About 200 have been placed in the U. S. so far. More than 500 others have been screened in Vienna, and 60% were found to be of sufficiently high caliber for entry into the U. S. Folders are available on them. The legal difficulties involved in placing these refugees in government positions were mentioned. The Food and Nutrition Board is studying protein and fat nutrition, and has published reports on various aspects of pesticides in food production and the use of chemical additives in foods. The need for a symposium on the uptake of organic materials by higher plants and by cells was suggested. A bill appropriating $280,000 for research on the effect of pesticides on fish and wildlife is before Congress. The Division passed a resolution urging intensified research on the effects of pesticides and growth regulators on plants, animals and man. Summary reports of 6 committees and a report to the public on the biological effects of atomic radiation have been published. An International Conference on Scientific Information is to be held in Washington in 1958. It will be sponsored by the NAS-NRC and the American Documentation Institute. As part of it there will be a discussion of problems of communication between biologists. The Institute of Laboratory Animal Resources has developed a set of minimum standards for the commercial production of random-bred and inbred laboratory mice, and plans to do the same for other laboratory animals. Its Committee on Procurement of Animals from Nature is being reorganized (the Representative is a member of the new committee). The second edition of its Handbook of Laboratory Animals is in preparation.

The "Handbook of Toxicology" will appear in 5 volumes, of which Vol. III (Pesticides) will have 3 parts of about 500 pages each. Other handbooks in preparation are "Standard Values in Circulation and Respiration", "Handbook of Aquatic Biology", "Standard Values in Biochemical Composition, Structure, and Activity", and "Standard Values in Growth, Reproduction and Life Histories". A special Committee on Renewable Natural Resources has issued its final, "tentative" report. It is not to be published, but a new, standing committee is to be appointed as the result of the Special Committee's recommendation. The annual report on fellowship activities under the aegis of the NAS-NRC was discussed at length. The Division of Biology and Agriculture makes nominations for National Science Foundation, Fulbright and other fellowships.

A. C. Simonpietri of the Office of International Relations reported that it is no longer difficult to get visas for Russian scientists to visit the U. S. The Chemical-Biological Coordination Center has been discontinued because some of its supporting agencies were no longer able to furnish funds. A Toxicological Information Center has been established in the NAS-NRC Division of Chemistry and Chemical Technology. Its director, Harry W. Hays, reported on its plans. It is financed by the Army, Navy, Air Force and Atomic Energy Commission. It does not intend to sponsor research projects. At first it will give advice and information to the Armed Forces, but it hopes to expand its activities later. The utilization of retired scientists was discussed. The American Physiological Society had made a pilot study of the problem, and its report will be distributed to members of the Division later. The following reports of committees of the Agricultural Board have been published during the past year: "The Fluorosis Problem in Livestock Production", "A Review of Bloat in Ruminants", "Composition of Concentrate By-Product Feeding Stuffs", and "Nutrient Requirements of Dairy Cattle". The Agricultural Research Institute now has 70 Class A members (industrial organization) and 97 Class B members (scientific societies, federal agencies, state agricultural experiment stations, etc.), of which the American Society of Parasitologists is one.

The report was accepted and ordered placed on file.

4. *To the Agricultural Research Institute* (*C. M. Herman*): Since our last annual meeting the A.R.I. has met twice, October 15 and 16, 1956 and October 14 and 15, 1957. The Representative attended all sessions at both meetings. At neither of these meetings was much attention devoted to parasitology.

Prior to the 1956 meeting, President Cram appointed a committee to assist in the preparation of proposals in the field of parasitology for presentation to the A.R.I. Three proposals were prepared and last year were approved by Council. They concerned further support of publication of a subject index of the Index-Catalogue of Medical and Veterinary Zoology, support of translation of foreign (particularly Russian) parasitological literature, and development of a reporting program on occurrence of disease in domestic and wild animals. Due to death of

the chairman of the A.R.I. Committee on Animal Health, no action has as yet been taken on these proposals. This committee is scheduled to meet in Chicago next December 1 and our proposals are included in the agenda.

The report was accepted and ordered placed on file.

5. *To National Council on Trichinosis (W. H. Wright)*: No report as there had been no meeting of that Council since our Storrs meeting.

6. *To National Register of Scientific and Technical Personnel (J. C. Larsh)*: No report since the Representative has received no communication from the Register since his appointment.

IV. Reports of Special Committees

1. *Committee on Constitutional Revision (A. O. Foster, Chr., C. Huff, P. E. Thompson, A. C. Walton, M. Young)*: Pursuant to proposals of the interim report to Council at its last meeting on August 26, 1956, the final report of the Subject Committee was transmitted to the Secretary on April 24, 1957. The Secretary sent reproductions of the proposed Instrument of Charter and By-Laws to members of Council on May 1. About 10 days later, the Instrument having received the required two-thirds approval of Council, a copy of the Instrument was mailed by the Secretary to each member of the Society in connection with the announcement of this 32nd Annual Meeting.

Other than typographical errors on pages 1, 2, 5, and 6, which can readily be rectified, there are a few items that should properly be placed before Council under items 6(a), 7(j), and 7(k) of the Agenda of this 47th Council meeting.

The Special Committee technically ceased to exist with the rendering of its report and the acceptance of the report by Council.

The report was accepted and ordered placed on file.

2. *Committee on Public Relations (H. W. Brown, Chr., A. C. Cuckler, B. H. Kean)*: An extensive correspondence was carried on during the year with Dr. Walsh McDermott who has been very active in U. S. Budget meetings concerning the Institute for Allergy and Infectious Diseases. The Committee also wrote letters in support of the new budget to Senator Lister Hill, Chairman, The Senate Committee on Labor and Public Welfare and to the Honorable John E. Fogarty, Chairman, Labor and Health, Education and Welfare Subcommittee. It was agreed among the representatives of the several scientific societies involved that Dr. Walsh McDermott and Dr. Rene Dubos should represent the group at the Congressional Hearings. This they did with considerable success. Both Houses proposed an increase of $4,000,000.00 for the Institute of Allergy and Infectious Diseases.

The report was accepted and ordered placed on file.

3. *Committee on Cumulative Index for the Journal of Parasitology, Vols. 26* through *40 (W. S. Bailey)*: Taking cognizance of the improved status of the funds in the office of the Custodian of Back Issues, Dr. Bailey proposed that Council authorize him to apply through the A.I.B.S. for sufficient funds from the National Science Foundation, or other sources that the A.I.B.S. might suggest, to defray one-half of the cost of publishing the Quindex with the other half of the cost to be defrayed from funds in the office of the Custodian of Back Issues.

This proposal was approved by the Council.

4. *Committee on Translations of Russian Works on Helminthology (K. C. Kates, Chr., G. Anastos, E. C. Dougherty, M. Doss)*: The Committee channeled its activities along the following lines with the indicated results: (1) Explored the feasibility of commercial translations; the latter proved to be prohibitively expensive. (2) Encouraged the development of a U.S.D.A. Translation Service; this has received general approval pending the allocation of funds. (3) In cooperation with the N.I.H. Translation Program, transmitted a proposal that 30 pages of the Journal of Parasitology be devoted to translations of selected articles; this proposal was rejected by the Society officials since it would proportionately defer the publication of original articles.

The Committee made the following recommendations: (1) That the Society through the Council strongly urge that all American institutions of higher learning, which offer graduate training in parasitology, require graduate students to obtain basic training in the Russian language. (2) That the Society through its Council urge some appropriate agency, such as the A.I.B.S. or National Science Foundation, to allocate funds for the preparation and publication of a comprehensive Russian-English Biological Dictionary. (3) That a small item somewhat as follows be published from time to time in a conspicuous place in the Journal of Parasitology. "It is requested that all members of the Society cooperate in making available to others translations of foreign parasitological publications, particularly those from Russian and other Slavic languages. It is suggested that this can best be done as follows: (a) Send one legible copy to the *Special Libraries Association Translation Pool, John Crerar Library, 86*

East Randolph Street, Chicago 1, Illinois (titles of available translations are published in 'Translation Monthly' by the SLAT pool, which also sells microfilms and photostats of translations at a nominal price). (b) Send one copy, if possible, to the *Index Catalogue of Medical and Veterinary Zoology, Beltsville Parasitology Laboratory, Beltsville, Maryland,* for its files and for citation in the published Index."

The Committee listed translations of 7 monographs on helminthology and 2 on ticks that have recently become available, and indicated how copies of these may be obtained.

No action was taken by the Council on the Committee's recommendations.

The report was gratefully accepted and ordered placed on file.

5. *Committee on Honorary Members (L. Olivier, Chr., M. Fallis, R. M. Lewert, H. W. Manter, R. B. McGhee, C. P. Read)*: Alternative recommendations were made pending disposition of the proposed new Constitution by the Society. With the adoption of the new Constitution the following recommendations became effective. As Emeritus Life Members; Doctors Fred Corry Bishopp, Asa Crawford Chandler, William Walter Cort, George Roger La Rue, and Horace Wesley Stunkard. As Honorary Members; Doctor Robert Philippe Dollfus, Professor Robert Thomson Leiper, and Dr. Satyu Yamaguti.

The Committee further recommended that an appropriate certificate and letter of notification be presented to each person that has been elected to honorary status by the Society. It also submitted a proposed model certificate along with an estimate of its cost.

The Council gratefully accepted the report and ordered that it be filed.

6. *Committee on Career Booklet and Placement Bureau (D. V. Moore, Chr., D. W. Micks, G. L. Hoffman)*: In reply to a request by Dr. Tippo of the A.I.B.S. for material on Parasitology in a proposed A.I.B.S. statement on "Education and Career Guidance", the following outline was submitted: (a) Definition of parasitology, (b) Training required, (c) General areas in which investigations are conducted, (d) Available positions for qualified parasitologists, (e) References to existing career guidance booklets.

The Committee requested the opinion of Council on the item of a career booklet to be sponsored by the Society. It emerged from the discussion that the Society should sponsor a career booklet. The Council then requested that the President appoint a committee to prepare an appropriate booklet for free distribution to high school guidance teachers, and others, upon request.

The Committee, recalling that the Society has taken no action on the establishment of a Placement Bureau because this function was considered to be fulfilled by the A.I.B.S., noted that the latter does not carry a specific listing for Parasitologists in its Placement Bureau. Upon recommendation of the Committee, the Council instructed the Secretary to request a specific listing for Parasitologists in the A.I.B.S. Placement Bureau.

The report was gratefully accepted and ordered placed on file.

7. *Auditing Committee (F. J. Kruidenier, N. D. Levine)*: The final complete 1956 reports of the Treasurer, Custodian of the Endowment Fund, and Custodian of Back Issues were found to be correct. Attention of the officers of the Society was directed to the 1956 deficit spending of the Society as reported by the Treasurer.

The November 1, 1957 reports of the Treasurer and of the Custodian of Back Issues were found to be correct on the basis of the records submitted. (Auditing of the 1957 report of the Custodian of the Endowment Fund was not undertaken by the Auditing Committee since the validity of the records had been attested by two respected members of the Society, *supra*).

V. Old Business

1. *Constitutional Revision:* Even though the proposed new Constitution had been accepted by the Council when circulated by mail on May 1, 1957, further consideration indicated that Section 1 of Article V should be reworded as follows: Instead of "Annual membership dues shall be seven dollars, payable in advance on or before the last day of January. The amount of the dues may be changed by a majority vote of the Society upon recommendation by the Council", the preferred wording would be "Annual dues shall be payable in advance on or before the last day of January. The amount of the dues shall be determined by a majority vote of the Society upon recommendation by the Council". The Council agreed to recommend this change to the Society before the Society was asked to vote upon the proposed new Constitution.

The Council reviewed the full implications of the changes in the proposed new Constitution relative to the manner of nominating Society officers and recommended that the President review this item during the business meeting before asking for Society action on the new Instrument.

2. *Gratis Subscription to the Journal of Parasitology to the Library of Congress:* The Council voted not to give the Library of Congress copies of all back volumes of the Journal and not to place it on the free mailing list for future issues. The Council was unanimous in

desiring that the Library have all copies of the Journal but a negative decision was reached because donation is not understood to be the usual manner by which the Library obtains non-copyrighted scientific journals and because of the precedent that would be set for the Society.

VI. New Business

1. *Dues and Subscription Rates:* Contingent upon Society acceptance of the proposed new wording of Section 1 in Article V as outlined under constitutional revision above, the Council agreed to recommend to the Society that in 1959 the dues for members be increased to $8.00 per year, the non-member domestic subscription rate for the Journal be increased to $9.50 per year and the non-member foreign subscription rate be increased to $10.00 per year.

2. *Quindex Committee:* Council suggested that the President should appoint, when appropriate, a committee to develop plans and select personnel to provide for the new Quindex (Index for Vol. 26–40 of the Journal). The appropriate time would be when arrangements have been completed for the necessary funds for the publication costs.

3. *Election of New Members:* Information on 9 applicants was presented to the Council in writing by the Secretary. All of the applicants were elected to active membership.

4. *Nomination of Emeritus Members:* Contingent upon Society adoption of the proposed new Constitution, the Council nominated the 5 members proposed by the Committee on Honorary Members (*supra*) for emeritus membership, namely, Doctors F. C. Bishopp, A. C. Chandler, W. W. Cort, G. R. La Rue, and H. W. Stunkard.

5. *Nomination of Honorary Members:* Contingent upon Society adoption of the proposed new Constitution, the Council nominated the 3 members proposed by the Committee on Honorary Members to honorary membership, namely, Doctors R. Ph. Dollfus and S. Yamaguti, and Professor R. T. Leiper.

6. *Nominations to Elective Offices of the Society:* The Committee of Tellers (L. A. Stauber and M. M. Brooke) presented a tabulation of the 86 ballots returned by the membership-at-large. Guided by these returns, except in the case of ties, the Council, sitting as a Committee-of-the-Whole, prepared the following slate of nominees for presentation to the Society at the Annual Business Meeting:

President: A. C. Walton (President-Elect during 1957)
President-Elect: A. O. Foster (to succeed A. C. Walton)
Vice-President: R. M. Cable (to succeed A. McIntosh)
Secretary: Paul E. Thompson (2-year term to succeed himself)

Council Members-at-Large: Gordon H. Ball and Dale A. Porter (2 for 4-year terms to succeed R. M. Cable and W. Trager).

Alternate Council Member-at-Large: Paul P. Weinstein (To serve for the 3-year term through 1960 in the event A. O. Foster is elected President-Elect).

7. *Elections to Editorial Committee and Editorial Board:* The Council elected H. T. Dalmat to the Editorial Committee for the 2-year term 1958–1959 to replace B. Davis (who has gone abroad). The Council elected to the Editorial Board D. L. De Giusti, Q. M. Geiman, R. M. Lewert (3 for 4-year terms) to replace W. Balamuth, W. W. Cort, and H. W. Stunkard, whose terms had expired.

8. *Petition of Southern California Parasitologists as an Affiliate of the American Society of Parasitologists:* By appropriate petition as set forth under Article VI of the new proposed Constitution and by personal representation by Dr. Gordon H. Ball, the Southern California Parasitologists applied for recognition as an Affiliate of the American Society of Parasitologists. Subject to Society approval of the new Constitution, the Council wholeheartedly agreed to accept this petition.

9. *Public Relations Committee:* The Council voted that this committee which heretofore has been an *ad hoc* committee be made a standing committee, with the personnel of the committee to be appointed by the President.

10. *Research Award:* Dr. Paul C. Beaver proposed that the Society consider an award or prize for encouraging fundamental research on parasites and parasitism. The Council authorized the President to appoint a committee to study this proposal.

11. *Appointment of Representatives: To the A.A.A.S.:* (1 representative for 2-year term) to be appointed by the President. *To the 9th Pacific Science Congress:* L. E. Rozeboom and E. H. Sadun. *To the 1958 Colloquium on Nomenclature:* A. McIntosh, N. R. Stoll and G. W. Wharton. *To the A.I.B.S. Concerning Delegates to 15th International Congress of Zoology:* L. E. Rozeboom and C. P. Read. *To the 6th International Congress of Tropical Medicine and Malaria:* To be appointed by the President.

12. *Standing Committee Appointments:*

(a) *Visual Education and Instruction:* M. S. Ferguson, Chr., D. V. Moore, J. C. Swartzwelder (Committee continued with no change in personnel).

(b) *Nomenclature and Terminology:* To be named by the President.

(c) *In Memoriam:* To be named by the President.

(d) *Public Relations:* To be named by the President.

13. *Ad hoc Committee Appointments:*

(a) *Resolutions:* R. M. Cable, F. J. Kruidenier.

(b) *Awards:* To be named by the President.

(c) *Career Booklet:* To be named by the President.

(d) *Auditing:* To be named by the President.

(e) *To Nominate a Candidate for Treasurer:* Under the proposed new Constitution this committee is to be named by the Chairman of the Nominating Committee. The 2-year term of the incumbent expires in 1960.

(f) *To nominate an Editor for the Journal:* To be named by the President.

14. *Selection of Meeting Sites for 1958, 1959, 1960:* In recognition of the need for planning more than one year in advance, the Council made the following proposals.

1958 Meet with the A.I.B.S., University of Indiana, Bloomington, Indiana, August 24–28.

1959 Meet with the A.I.B.S., Pennsylvania State College, State College, Pennsylvania, last week in August.

1960 Meet with the American Society of Tropical Medicine and Hygiene, place and time to be worked out between representatives of the two Societies.

15. *Other Items:*

Owing to some recent uncertainty regarding financial reports being submitted in terms of a fixed fiscal year, the Council expressed the opinion that it is preferable that the reports correspond as closely as possible to the intervals between the appropriate successive annual meetings rather than to adhere to a fixed fiscal year.

President Otto transmitted descriptive information to the Council concerning the recently organized American Academy of Microbiology. No proposal was made and no action was taken by the Council.

Dr. H. E. Meleney presented the "Third Annual Report of the Louisiana State University Fellowship Program in Tropical Medicine and Parasitology" before the Council. He pointed out that this program will continue, under the direction of Dr. William Frye after September 1, on a somewhat broader basis. It is contemplated that faculty members and graduate students from both medical and non-medical institutions will be eligible for fellowships, and that fellowships of 6 to 12 months duration will be awarded. The Council expressed deep appreciation to Dr. Meleney for his efforts in behalf of the Program.

The Council voted to adjourn at 2:45 A.M., October 31, 1957.

Respectfully submitted,
/S/ Paul E. Thompson, Secretary

AMERICAN SOCIETY OF PARASITOLOGISTS
THIRTY-SECOND ANNUAL GENERAL BUSINESS MEETING
NOVEMBER 1, 1957

The General Business Meeting of the Society was called to order by President Otto at 1:30 P.M. following the annual luncheon held in the Crystal Ballroom of the Benjamin Franklin Hotel.

1. The Society was asked to vote upon the proposed new Constitution as circulated to the membership on May 8, 1957, with a modification in Section 1 of Article V to make it read as follows: *"Dues.* Annual membership dues shall be payable in advance on or before the last day of January. The amount of the dues shall be determined by a majority vote of the Society upon recommendation by the Council." The new Instrument was adopted by the Society without a dissenting vote.

2. The status of the membership and of the financial affairs of the Society were summarized briefly.

3. A brief report on the Journal was presented.

4. The report of the In Memoriam Committee and the wording of a telegram of condolence to Dr. George R. La Rue concerning the death of Mrs. La Rue that was sent on behalf of the Society and of its members individually were presented.

5. The possibilities of useful services by the A.I.B.S. to its affiliated societies in the form of reducing the clerical work of the Chairman of the Editorial Board, the Treasurer and the Secretary were reviewed.

6. The intention to prepare a career booklet on parasitology was announced.

7. An announcement was made that the Louisiana State University Fellowship Program

in Tropical Medicine and Parasitology was being continued with contemplated plans for an extension in scope. The Society gave a rising vote of appreciation to Dr. H. E. Meleney for his efforts in behalf of the Program.

8. The names of Council appointees to the Editorial Committee and to the Editorial Board were read.

9. Council presented the following nominees: As Emeritus Life Members, Doctors F. C. Bishopp, A. C. Chandler, W. W. Cort, G. R. La Rue, and H. W. Stunkard; as Honorary Members, Dr. R. P. Dollfus, Professor R. T. Leiper, and Dr. S. Yamaguti. On motions from the floor the Secretary was instructed to cast unanimous ballots for these nominees. The Secretary so voted. Plans were announced to present a certificate suitable for framing to all Emeritus Life and Honorary Members.

10. The slate of nominees as candidates for elective offices in the Society prepared by Council, primarily from the returns of membership balloting, was presented. On motions from the floor, the Secretary was instructed to cast unanimous ballots for the individuals so nominated to the various positions. The Secretary so voted.

11. Upon recommendation of Council, the Society voted beginning in 1959 to increase the membership dues to $8.00 and the Journal rates to $9.50 for domestic subscription and to $10.00 for foreign subscription.

12. Following a call for nominations from the floor, the Society elected Dr. Horace W. Stunkard as Chairman of the Nominating Committee.

13. Upon Society adoption of the new Constitution, it was announced that Council would be pleased to accept the petition of the Southern California Parasitologists to become an affiliate of the American Society of Parasitologists on the effective date of the new Instrument—January 1, 1958.

14. Council announced an intention to propose annual meeting plans several years in advance because of the obvious advantage to all concerned. Meeting plans for the next 3 years were announced as follows:

1958, with A.I.B.S., University of Indiana, Bloomington, Indiana, August 24–28.

1959, with A.I.B.S., Pennsylvania State University, State College, Pennsylvania, last week in August.

1960, with American Society of Tropical Medicine and Hygiene, Projected pending the development of plans for a suitable place and time by representatives of the two Societies.

15. A resolution was voted expressing sincere appreciation to the Local Committee, the American Society of Tropical Medicine and Hygiene, and to the Benjamin Franklin Hotel for their contributions to the success of the Thirty-Second Meeting of the Society.

16. The Society voted to adjourn at 2:30 P.M.

Respectfully submitted,
/s/ Paul E. Thompson, *Secretary*

THE AMERICAN SOCIETY OF PARASITOLOGISTS

Charter and By-Laws

CERTIFICATE OF INCORPORATION

We, the undersigned, all citizens of the United States, and a majority citizens of the District of Columbia, desiring to associate ourselves as a corporation pursuant to the provisions of Subchapter three (3), of Chapter eighteen (18), of the Code of Law for the District of Columbia, do hereby certify as follows:

First. The name of title by which this corporation shall be known in law shall be THE AMERICAN SOCIETY OF PARASITOLOGISTS.

Second. The term for which it is organized shall be in perpetuity.

Third. The particular business and objects of said corporation shall be to conduct a scientific society; to publish a scientific periodical; to collect subscriptions thereto; to collect funds for prizes, awards, and for other purposes; to make contracts; to acquire, hold, and dispose of real and personal property; to make investments; and to disburse collected funds for expenses, awards, and other purposes.

Fourth. The number of its trustees, directors, or managers for the first year of its existence shall be twelve (12).

In Testimony Whereof, we have this 22nd day of September, 1932, hereunto set our hands.

Maurice C. Hall (Seal)
Benjamin Schwartz (Seal)
E. W. Price (Seal)

BY-LAWS

A revision of Instruments published in March, 1925; March, 1926; December, 1938; April, 1940; December, 1941; December, 1943; and December, 1947; and of intervening and later amendments approved by the Society and/or the Council.

Article I. Name

The organization shall be known as THE AMERICAN SOCIETY OF PARASITOLOGISTS, hereinafter referred to as the Society.

Article II. Object

The Society fosters association of persons interested in parasitology and related sciences to improve teaching, promote investigation, and advance knowledge of these sciences.

Article III. Members

Section 1. *Classes.* Members shall be of three classes; namely, active, emeritus, and honorary.

Section 2. *Active Members.* Any person with educational or other qualification who subscribes to the Object of the Society may be elected an Active Member.

A candidate for active membership shall send to the Secretary a duly executed and signed application accompanied by remittance for one year's dues.

An affirmative vote of all Council members at a regular meeting or properly called special meeting, or of all that reply to mail ballot within 30 days, with due regard to quorum, shall be necessary for election.

Active Members whose current dues are paid are *members in good standing* and shall enjoy all rights, privileges, and responsibilities of membership in the Society, including subscription to the Society's publications.

Active Members may temporarily be made Inactive Members without prejudice by reason of military service, residence abroad, hardship, or other exigency, provided a request for this status, wth reason, is made to the Secretary and approved by the Council. Inactive Members shall not pay dues, vote, receive the Society's publications, or hold office but will automatically be restored to the status of Active Members upon resumption of payment of dues.

Active Members whose current dues are unpaid shall be regarded as *delinquent* and shall not receive the Society's publications or hold office. *Delinquent members* shall be notified by the Treasurer, upon second billing, that those whose dues are in arrears for two years will be dropped from the rolls of the Society.

Individuals dropped from membership for non-payment of dues, or who have resigned, may be automatically reinstated by payment of current dues and all dues in arrears. Otherwise, an applicant for reinstatement to membership must apply for election as a new member.

Section 3. *Emeritus Members.* Distinguished parasitologists who have retired from active professional duty and who have rendered notable service to the Society for a period of not less than twenty years may be elected Emeritus Members.

The number of Emeritus Members shall not exceed one per cent of the number of Active Members at the time of election.

Emeritus Members shall be exempted from payment of dues but shall be accorded all rights and privileges of Active Members, including subscription to the Society's publications for so long as they indicate desire to have same.

Election of Emeritus Members shall require the unanimous recommendation of Council members at a regular meeting and a three-fourths affirmative vote of Active Members of the Society present at a regular business meeting.

Nominees may be proposed to the Council by written communication to the Secretary.

Section 4. *Honorary Members.* Any person who has attained exceptional distinction in parasitology and who is not an Active Member of the Society may be elected an Honorary Member.

The number of Honorary Members shall not exceed fifteen and not more than three shall be elected during any one year.

Honorary Members shall pay no dues and shall not be eligible to hold office. They may elect to pay dues assessed for active membership, in which event they shall be accorded the privileges of Active Members, including subscription to the Society's publications.

Election of Honorary Members shall be by the same procedure as for Emeritus Members.

ARTICLE IV. ADMINISTRATION

Section 1. *Officers and Council.* The Society shall be governed by a Council of fifteen officers of the Society; namely: the President, President-Elect, and Vice President, whose terms of office shall be one year; the Secretary and the Treasurer, whose terms shall be two years; eight Members-at-Large of the Council, grouped in four classes of two each, whose terms shall be four years and staggered in such manner that there are two regular vacancies to be filled each year; and the Editor and the Custodian. The five first-named officers shall simultaneously hold the same offices on the Council and in the Society.

The Treasurer, or the Treasurer and the President jointly, as and when directed by the Council, shall select depositories for the Society's monies, assign securities and investments belonging to the Society or controlled by it, and endorse financial and legal papers in behalf of the Society, except that the Treasurer shall have the authority and responsibility to receive dues and other obligations owing to the Society and to pay current and other expenses authorized by the Council.

Section 2. *Order of Succession and Vacancies.* The order of authority, responsibility, and succession among Officers of the Society and Council, becoming effective whenever an officer does not or cannot fulfill the ordinary duties and responsibilities, whether administrative or not, of his office shall be as named above, excluding the President-Elect, followed by Council Members-at-Large by classes according to seniority and alphabetical order of surnames. Service by succession shall be temporary pending action by the Council or Society, and shall not create a further vacancy in any office.

Except as provided in Section 7 and excluding the office of President-Elect, a vacancy occurring in any office, elective or other, may be filled by designation of the Council either for the remainder of the unexpired term of the incumbent or until the next regular or annual meeting of the Society.

Section 3. *Election of Officers. Nominating Committee.* The Society, at each regular or annual meeting, shall elect one of its members in good standing to serve as Chairman of the Nominating Committee for the ensuing year or until the next election of officers by the Society. The eight Council Members-at-Large shall comprise the remainer of the Committee.

The Nominating Committee shall meet at least once and properly complete its business prior to each regular or annual meeting of the Council. In the absence of the Chairman, the senior Council Member present shall preside. The Council shall determine compliance with these requirements and, in the event of non-compliance, shall itself sit as a nominating committee.

The Nominating Committee shall submit to the Society, at the regular or annual meeting of the latter, at least one nominee for each office to be filled. Nominations from the floor shall also be in order. When there are two or more nominees for any office, voting shall be by ballot.

At least 90 days prior to each annual meeting, the Secretary shall inform all Active and Emeritus Members of the offices to be filled and invite nominations for each, said nominations to be tallied by a Committee of Tellers whose report shall be made to the Chairman of the Nominating Committee.

The Nominating Committee shall be bound by nominations from the Membership-at-Large if a quorum of members have voted and a nominee has received a majority for any office, except that of Secretary or Treasurer. Candidates for the office of Secretary or Treasurer shall be proposed by a subcommittee appointed by the Chairman of the Nominating Committee.

Section 4. *Council.* The Council shall manage all affairs, funds, and properties of the Society and transact all business, except as otherwise provided. It shall have the right to delegate temporarily to members, special committees, or subcommittees, any responsibility or power with which it is, or normally would be, vested.

Section 5. *President.* The President shall preside at all meetings of the Society and Council; appoint all committees except as provided in Section 3; be an *ex officio* member of all committees except the Nominating Committee; and perform all duties that properly devolve upon the chief administrative and presiding officer of the Society.

Section 6. *President-Elect.* The President-Elect shall assume the office of President upon completion of his term of office and perform such other duties as the Society, Council, or President may assign to him or to the office of President-Elect.

Section 7. *Vice President.* The Vice President shall function in lieu of the President in the event of the latter's absence or inability for any reason to discharge or perform any duty or responsibility of his office or that ordinarily devolves upon it. He shall also perform such duties as the Society, Council, or President may specifically assign to him or to the office of Vice President.

Section 8. *Secretary.* The Secretary shall record the minutes of all meetings of the Society and Council, prepare the agenda for these meetings, keep accurate and complete reports of all officers and committees and of all business transacted by the Council and Society, maintain rolls of members of all categories, and perform such other duties as properly devolve upon his office.

Section 9. *Treasurer.* The Treasurer shall issue all statements of financial obligations owing to the Society and account for all funds, properties and disbursements, except as provided in Art. VII, Sec. 4; and shall pay all bills owed by the Society and authorized by the Council.

He shall maintain a separate account of all securities, monies, earnings, increments, and disbursements of the Endowment Fund.

He shall submit an annual financial statement to the Council and to the Society which shall be completed to the most convenient recent date prior to the time of reporting and cover the period from the closing date of the last such report.

Section 10. *General Responsibilities of Officers.* All official duties and responsibilities not expressly defined shall devolve upon those officers who customarily are charged with such duties and responsibilities.

No officer shall relinquish his office until and unless his duties and responsibilities are assumed by a duly elected or appointed successor.

The Council may redefine at any time the duties and responsibilities of an officer.

Article V. Finances

Section 1. *Dues.* Annual membership dues shall be payable in advance on or before the last day of January. The amount of the dues shall be determined by a majority vote of the Society upon recommendation by the Council.

Section 2. *Auditing.* Reports of the Treasurer and Custodian shall be audited either by a Public Acountant or an Auditing Committee of three members, the method to be determined annually by the Council.

Section 3. *Bonding.* The Treasurer and Custodian shall be bonded for a sum determined annually by the Council.

Section 4. *Expenses of Officers.* Officers who receive allotments of funds for conduct of their offices shall keep an itemized account of disbursements and submit same to the Treasurer in time for inclusion in the regular or annual statement by the Treasurer. Unexpended balances shall revert to the Treasury.

Such allotments may be made only by the Council and are in addition to fixed sums that this body may authorize for payment to certain officers in lieu of expenses for attending meetings.

Article VI. Regional Branches and Affiliated Organizations

Section 1. *Establishment.* Regional branches of the Society may be established and existing organizations with an interest in the disciplines of parasitology may become affiliated with the Society, by action of the Council, on a petition by 10 Society members in good standing and residing within the geographic boundaries of the branch or organization. The boundaries shall be indicated in the petition to the Council and any subsequent changes shall be made only with permission of the Council. The Council shall exercise no jurisdiction over the name of any branch or affiliated organization.

Section 2. *Sovereignty.* Regional branches or affiliated organizations shall be free to elect officers, collect dues and govern themselves, subject only to compliance with provisions stated herein. Policies set forth in the Constitution and By-Laws of such groups shall be in harmony with the policies, including the Charter and By-Laws, of the Society. A copy of the Constitution and By-Laws of regional or affiliated organizations shall be filed with the Secretary.

Section 3. *Members.* Membership in regional branches or affiliated organizations shall not be restricted to members of the Society.

Section 4. *Officers.* All officers of regional branches or affiliated organizations shall be Society members in good standing. A list of officers shall be filed with the Secretary within 3 weeks after their election.

Secton 5. *Representation.* Regional branches or affiliated organizations with 25 or more Society members in good standing may appoint or elect a representative to the Council. This representative shall be free to participate in the discussions of the Council but shall be a non-voting member of that body.

Section 6. *Reports.* Regional branches or affiliated organizations shall submit to the Council, through the Secretary, a short, concise, annual report of their activities and copies of

their programs of meetings, announcements, and other activities. This information must be in the hands of the Secretary prior to the annual Council meeting.

Section 7. *Meetings.* Regional branches or affiliated organizations may hold meetings at their own discretion provided that such meetings do not conflict with the annual meeting of the Society.

Section 8. *Use of Society Publications.* Announcements and programs of meetings held by regional branches or affiliated organizations may be published in THE JOURNAL OF PARASITOLOGY but the cost of publication must be borne by the branch or organization, unless otherwise determined by the Editor and Council.

Section 9. *Dissolution.* The Council may at any time, for good cause, dissolve or relinquish affiliation with a regional branch or affiliated organization.

Article VII. Publications

Section 1. *Society Publications.* The official publication of the Society is THE JOURNAL OF PARASITOLOGY, a property of the Society.

There may be such other publications as the Council and Society authorize.

Section 2. *Editorial Board.* All affairs related to Society publications, except as provided otherwise, shall be managed by an Editorial Board consisting of an Editor and two Associate Editors, who shall be appointed by the Council for terms of five years each, and twelve Assistant Editors, grouped in four classes of three each who shall be appointed by the Council for four-year terms staggered in such manner that there are three regular new appointees each year. There shall be such other assisting personnel as the Council deems essential.

Members of the Editorial Board shall be Society members in good standing and shall represent to the fullest practicable degree the scientific interests and geographical distribution of the Society's members.

Section 3. *Editor.* The Editor shall have responsibility and authority for formulating publication policies; for deciding format and content; for reviewing, editing, and determining the acceptability of notes and papers offered for publication; for determining charges to authors and advertisers; for conducting negotiations with authors, advertisers, and publishers; for recommending to Council suitable nominees to fill vacancies on the Editorial Board; and for performing all duties that ordinarily devolve upon an Editor.

In the performance of these duties, the Editor will rely upon the active cooperation of the Editorial Board but may also seek advice and assistance from any member of the Society.

Neither the Editorial Board nor any member thereof shall be charged with responsibility or authority that abridge the duty of the Treasurer to receive and disburse Society funds or that transcend the obligation of Council to guard the interests of the Society and protect its welfare.

The Editor shall report fully to the Council on activities, needs, and problems regarding the management and status of the Society's publications.

Section 4. *Custodian.* There shall be a Custodian of back issues of Society publications, appointed by the Council for a term of three years, who shall maintain repositories and inventories of back issues of THE JOURNAL OF PARASITOLOGY and other Society publications, and be responsible for all activities and negotiations related to the sale, reproduction, and purchase of back issues.

The Custodian shall report to the Council on expenses, income, inventories, sales, purchases, reproductions, activities, needs, and problems regarding the care and status of back issues.

Article VIII. Rules of Order

Section 1. *Application and Procedure.* The Society, its officially constituted committees, and internal bodies of members, its officers and members shall observe in their conduct the procedures defined in these By-Laws, and in the special and general rules of order of the Society.

Section 2. *Special Rules of Order.*

(i) *Quorum.* One hundred members in good standing shall be a quorum of the Society. A majority of the members of the Council shall be a quorum of that body.

(ii) *The Society Year.* The Society year shall be the calendar year, except as implied otherwise (e.g. Treasurer's report, Nominating Committee).

(iii) *Review by Society of Council Action.* No action by the Council shall abridge the right of the Society to debate any question or to review any action or decision by the Council, and any such action or decision may be changed by an affirmative vote of two-thirds of the members at a duly called business meeting or of those replying within 60 days to mail ballots issued by the Secretary, provided votes are cast by a quorum of the members and provided

also that Society action does not involve commitment, obligation, or payment of funds on which the Council has already acted in accordance with its duly invested authority and responsibility.

(iv) *Change in Status of Honorary Members.* Honorary Life Members and Foreign Honorary Members, elected prior to January 1, 1958, shall be automatically transferred to the status of Emeritus Members and Honorary Members, respectively.

Section 3. *General Rules of Order.* Robert's Rules of Order, Revised, shall govern deliberations in all cases where they are applicable and not inconsistent with these By-Laws or the Special Rules of Order of the Society.

Article IX. Amendments

These By-Laws may be amended at any regular business meeting by a two-thirds vote of the Society, provided members have been given thirty days' notice of intention to amend and a copy of the proposed amendment, or by mail ballot recording an affirmative vote of two-thirds of the members responding within 60 days, with due regard to quorum.

All amendments, whether relating to addition, repeal, or alteration, and dates of their adoption shall be recorded seriatim by the Secretary and filed with a copy of these By-Laws.

RESEARCH NOTE

AN IMPROVED METHOD FOR PURIFYING BALANTIDIA

One of the problems in handling intestinal ciliates such as species of *Balantidium*, is that of separating the animals from intestinal debris. Nelson (Am. J. Trop. Med. **20**: 731–745, 1940) described a washing device consisting of two 5-inch lengths of glass tubing ¼" in diameter connected by rubber tubing. The lower piece was bent into a V and the upper one had a constriction 1 inch from the upper end. To the free end of the V-tube another piece of rubber tubing with a pinch clamp was attached. A loose plug of absorbent cotton was placed above the constriction in the straight tube. After filling the device with washing fluid, the ciliate-containing material was placed above the cotton plug. The balantidia migrated through the cotton and accumulated at the bottom of the upright tube. By clamping the connecting tube the ciliates could be withdrawn with a pipette through the free arm of the V-shaped tube.

The newer method is based in part on suggestions by personal communication from N. D. Levine and worked out in connection with studies supported by Public Health grants R. G. 4527 and E-999 (C).

In the improved device (Fig. 1), the upper tube is a cylinder about 2 cm in diameter with a capacity of about 20 ml. This is tapered and fused with a smaller tube about 7 mm in diameter. After the system is filled with Ringer's fluid, 2 or 3 loose cotton filters are placed in the narrow tube above the rubber connecting tube and a larger cotton filter is installed at the bottom of the larger cylinder. These filters provide for a much more complete separation of the ciliates from the debris.

The intestinal contents (*e. g.* from the pig) are diluted with Ringer's fluid 1 to 3 and filtered through gauze into a beer glass or other conical container. This, with its contents, is placed in an incubator at 37° C for 20 to 30 minutes. The sediment will contain the ciliates and the finer particles coming through the gauze. The supernatant is decanted and replaced by fresh Ringer's fluid. The sedimentation, decantation and dilution procedure is repeated 3 times and serves to remove colloidal particles which might clog the cotton filters. After the last sedimentation and decantation, and after thorough stirring, the sediment and residual Ringer's fluid are placed in the upper part of the separation apparatus. The balantidia migrate through the cotton filters and accumulate at the bottom of the upright tube. With the upper tube closed off by the clamp, the lower clamp can be opened and the ciliates withdrawn with a pipette through the free arm of the V-tube, as with Nelson's device.

The entire apparatus is attached with scotch tape to a glass plate 38 × 17.5 cm. For faster collecting and greater numbers, 2 or 3 such devices can be attached to 1 side of the glass plate (Fig. 1) and an equal number could be placed on the other side of the plate.

For *Balantidium coli* and *B. caviae* the plates with the tubes containing the ciliates should be kept in an incubator at 37° C. *B. caviae* is more sluggish and does not accumulate so rapidly; also it does not survive so long in Ringer's solution, hence the concentrated material should be used as quickly as possible. *B. coli* from the pig will live in the "purified" condition at 37° C for as long as 24 hours to several days without noticeable changes in activity. Balantidia from cold-blooded hosts should, of course, be handled at room temperature.

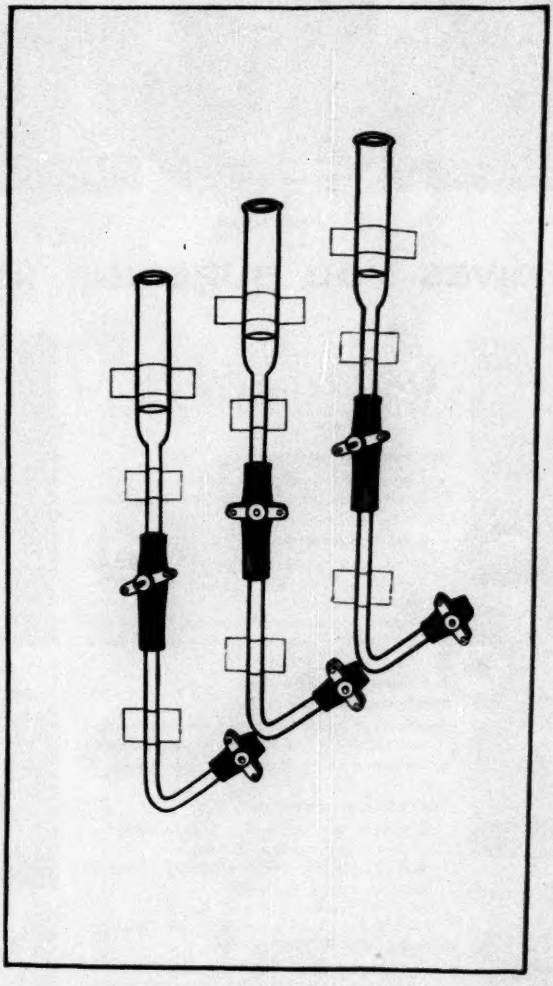
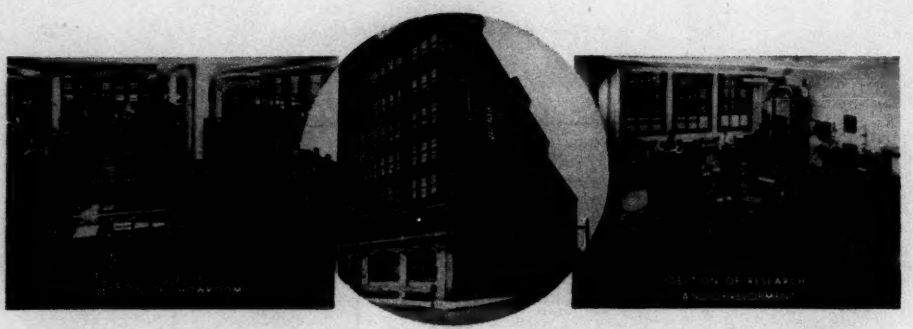


FIGURE 1

The "purified" and concentrated ciliates may be used for demonstrations, for studies of living animals, as on a depression slide, for fixing and staining, for inoculation into cultures and for inoculation into appropriate animals for serological studies.—SERHIJ KRASCHENINNIKOW, *Zoological Laboratory, University of Pennsylvania, Philadelphia, Pa.*



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